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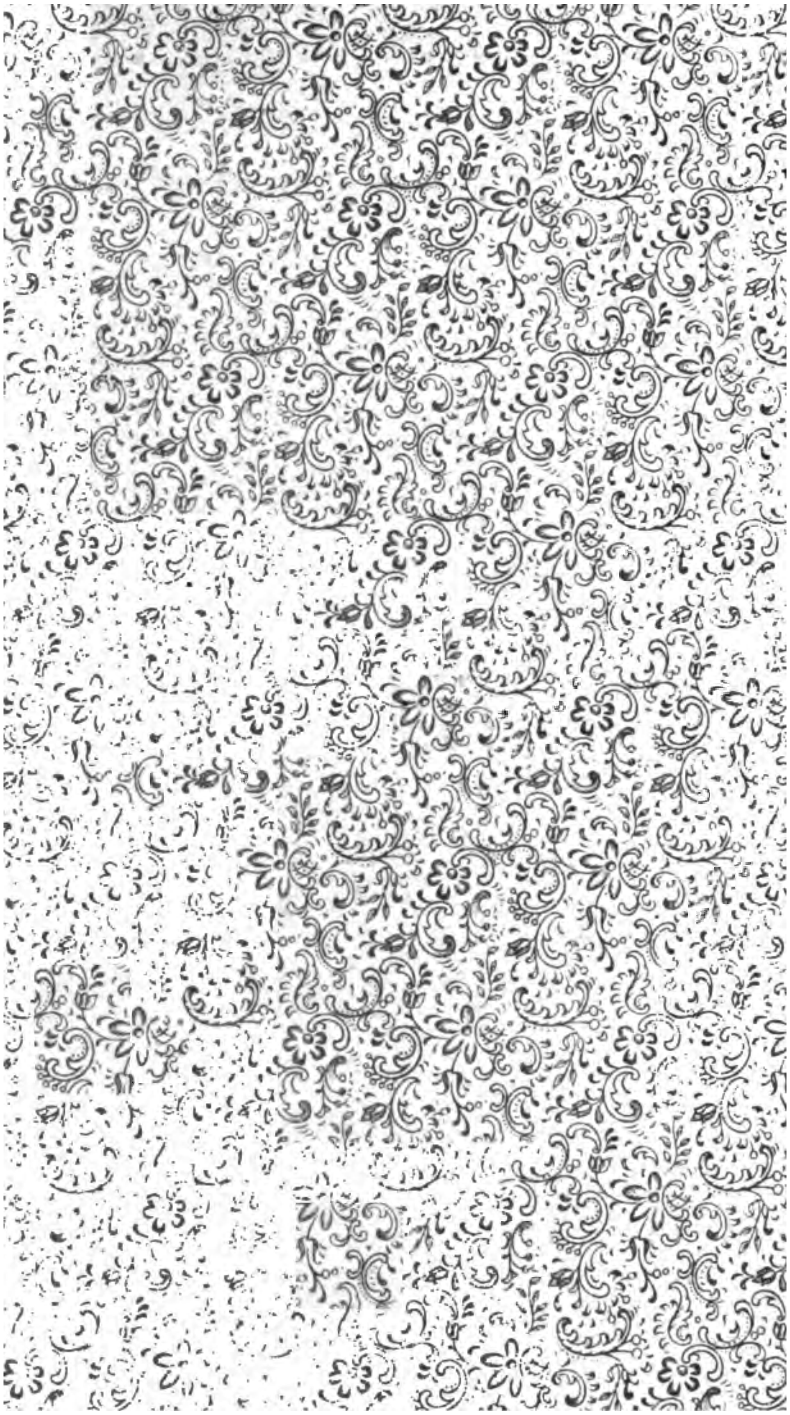

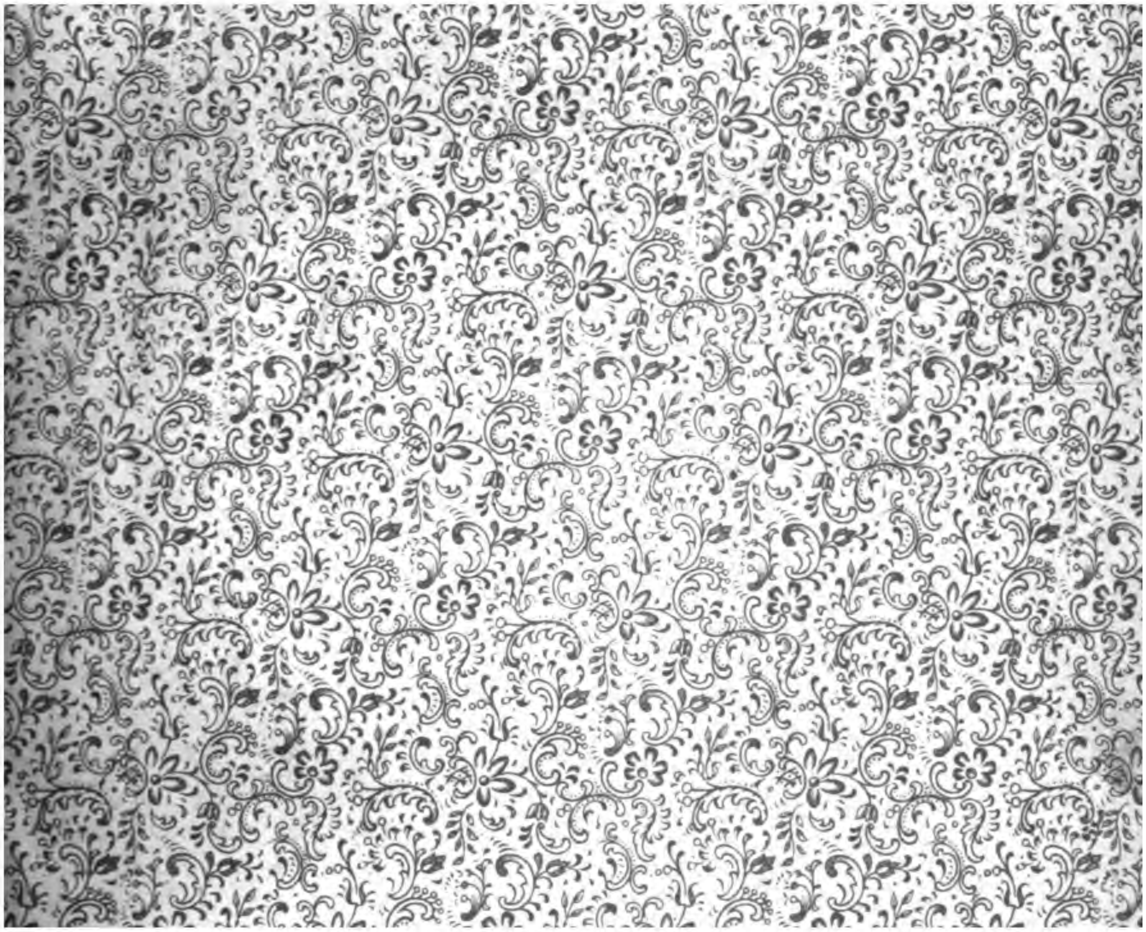

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1921, xviii, pp. 275–277.

139 (1721)

Studies on experimental rickets, IV.
Cod liver oil as contrasted with butter fat in the protection against the effects of insufficient calcium in the diet.

By E. V. McCOLLUM, NINA SIMMONDS, P. G. SHIPLEY
and E. A. PARK.

[*From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, and from the Department of Pediatrics, Johns Hopkins University, Baltimore.*]

In our experimental work we have made observations which demonstrate in a striking way the differences in the effectiveness of cod liver oil as contrasted with butter fat in influencing the rate and extent of growth, and their effects on the histological structure of the bones. This is well illustrated by the results of restricting young rats to the following diet:

Wheat	30.0
Maize	19.5
Rice (polished)	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak	10.0
$NaCl$	1.0
$NaHCO_3$	1.5
Cod liver oil	3.0

This diet induces fairly good growth and fertility during at least 8 to 10 months, notwithstanding its deficiency in calcium. The calcium content of this food mixture is 0.059 gms. per 100. The optimum calcium content of this diet is reached when about 1.5 per cent. of calcium carbonate is added. The phosphorus content of this mixture is 0.3546 gms. per 100, and is not far from the optimum content. This food mixture is slightly alkaline owing to its content of sodium bicarbonate.

This food mixture with butter fat to the extent of 3, 10, or 20 per cent. of the food mixture fails to induce an appreciable amount of growth. With 20 per cent. of butter fat the animals gain but a few grams in weight, look very inferior, become short and stocky, and are rough coated. They usually die within a few months.

This diet when 10 per cent. of casein is added, but without the addition of either butter fat or cod liver oil, causes pathological changes characteristic of rickets. With small amounts of cod liver oil (3 per cent.) no rachitic changes are seen in the bones. Even 20 per cent. of butter fat fails to effectively direct the growth processes in the bones toward the normal condition.

This food mixture containing 3 per cent. of butter fat and 1.5 per cent. of calcium carbonate is a highly satisfactory diet for the promotion of growth to the full adult size, the maintenance of high fertility throughout the breeding period in the females and the successful nutrition of the young by the mothers. Even a fifth generation in a family confined to this diet showed no signs of physical deterioration. Without the calcium addition, but with 3 per cent. of butter fat, very little growth can take place.

The striking differences between the nutritive properties of cod liver oil as contrasted with butter fat we have up to the present time been able to demonstrate in so satisfactory a manner only with diets in which the phosphorus content is approximately the optimum, and with the calcium content distinctly below the optimum. The results of feeding these two fats with a diet similar to that described but in which the phosphorus content is low and the calcium varies from low to high, we shall discuss in a forthcoming paper.

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140 (1722)

Studies on experimental rickets, V. The production of rickets by means of a diet faulty in only two respects.

By P. G. SHIPLEY, E. A. PARK, E. V. McCOLLUM and NINA SIMMONDS.

[From the Department of Pediatrics, and from the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.]

The following diet, when fed to the young rat, in a comparatively short time (three to five weeks) produces rickets. The diet is composed of:

Rolled oats	40.0
Gelatin	10.0
Wheat glutin	7.0
Sodium chloride	1.0
Potassium chloride	1.0
Calcium carbonate	2.0
Dextrin	39.0

This diet is extremely poor in fat-soluble A, the anti-xerophthalmic substance. Young rats develop xerophthalmia when placed upon it in from four to five weeks. Its proteins are of good quality and are supplied in abundance (21 per cent.). The phosphorus content is relatively low (0.209 gms. per 100 gms. of the food mixture). The calcium content is approximately the optimum.

The gross evidences of rickets in the rats eating the faulty ration were briefly these: deformities of the thorax consisting in flattening or hollowing along the line of costochondral junctions; pigeon breast; enlargement and distortion of the costochondral junctions; fractures of the ribs; enlargements of the ends of all the long bones; diminished resistance of the bone to cutting; great diminution in the tensile strength; and finally, the presence of a zone of a white or pale yellow color, between the cartilage and the shaft, visible to the naked eye, the rachitic metaphysis.

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STUDIES ON EXPERIMENTAL RICKETS.

VI. THE EFFECTS ON GROWING RATS OF DIETS DEFICIENT IN CALCIUM.

BY E. V. McCOLLUM AND NINA SIMMONDS

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore),

AND

P. G. SHIPLEY AND E. A. PARK

(From the Department of Pediatrics, Johns Hopkins University, Baltimore)

(Received for publication, June 6th, 1921.)

PLATES XXV TO XXIX. (FIGURES 1–10.)

PART I.

In the first paper of this series (2) we placed on record a series of faulty diets, which, when fed to the growing rat, caused pathological conditions of different kinds to develop in the skeleton. The pathological condition induced in the bones by some of the diets bore marked resemblances to those which characterize rickets in the human being. The diets were, relatively speaking, simple in their composition; their faults were deficiencies in fat-soluble A, calcium, phosphorus, excess of calcium, etc. In an accompanying paper (3) we brought forward experimental proof of the remarkable influence exerted by cod liver oil on the deposition of lime salts in the bone of the rat. In rats which had lost the power to deposit lime salts in the epiphyseal cartilage as the result of the prolonged administration of certain faulty diets, the addition of cod liver oil to the diet without other change quickly restored the lost power to the organism. In subsequent communications we have brought forward evidence to indicate that in the presence of an insufficient amount of an organic factor in the diet—which provisionally we call fat-soluble A—a lowering of the phosphate content of the diet below a certain level—the calcium being present in optimal or above the optimal proportion, and all other constituents of the diet being in a proportion not far from the optimal—may cause changes of a pathological nature to develop in the skeleton which have all the gross and histological characteristics of rickets. When the deficiency in the phosphate content of the diet was completely compensated for, however, by the addition of the phosphate ion (the deficiency in fat-soluble A still existing) the changes which developed in the skeleton were not those of rickets, but of osteoporosis. Our experiments have shown over and over again that bone is a labile tissue, and that the character of its growth can be easily influenced through the diet, and that gross deformities of the skeleton, however much they may resemble those of rickets in the human being, can not be accepted as proof of rickets. The proof of the production of rickets must be sought in the histological changes in the bones themselves.

In papers which are forthcoming we shall attempt to describe the changes which result from restricting young rats to diets in which the fault is twofold; e.g., low calcium with low fat-soluble A, high calcium with low fat-soluble A, and low calcium with liberal amounts of cod liver oil (high fat-soluble A?). We shall also discuss the effects on the young rat of diets low in calcium, but supplemented with the vegetable oils and varying amounts of butter fat, and of high calcium with fats of various origins. In the present communication we describe the effects on growth and the changes in the skeleton which result from restricting the intake of calcium, the diet being in all other respects suitably constituted.

In interpreting the data presented in these papers it should be appreciated that modern researches in the field of nutrition have established the validity of the working hypothesis proposed by McCollum and Davis (5) in 1915. According to this hypothesis, a diet, to be satisfactory for nutrition during growth or for the maintenance of well-being in the adult over a prolonged period, must furnish the following elements or complexes. It must contain proteins in satisfactory amount. The amount of protein necessary will depend upon its quality, and its quality in turn is the result of its composition with respect to the several amino-acids which are indispensable for the construction or repair of body tissues. Proteins from different sources differ greatly in their constitution in these respects. Nine inorganic elements in appropriate compounds are indispensable. These are sodium, potassium, magnesium, calcium, iron, phosphorus, chlorine, sulphur and iodine. With the exception of sulphur, which must be supplied in the form of the organic complex, cystine, these inorganic elements are all readily assimilated and utilized in the form of mineral salts, such as the chlorides and phosphates of the alkalies and alkaline earths. The food must furnish sufficient energy-yielding substances in the form of carbohydrates or fats. The one indispensable complex in this class is glucose. Either glucose or a substance capable of yielding it must be present. The diet must likewise furnish sufficient water, and in the rat at least two still uncharacterized dietary factors now ordinarily called "vitamines." In man a third factor in this class, the antiscorbutic substance, is essential.

Much of the research in the laboratory of the Department of Chemical Hygiene during the past few years has been directed toward the study of the quality of each dietary factor in each of our more important foodstuffs of animal and vegetable origin. It has been accomplished through the application of our biological method for the analysis of a food. Following the studies of the properties of individual dietary constituents numerous experiments were carried out to determine the extent to which two or more natural foods when combined enhance each other's protein values, and mutually supplement each other's deficiencies (6).

In the course of the investigations last referred to we employed a number of diets of considerable complexity for the purpose of determining whether, when the selection was limited to such vegetable products as are functionally storage tissues of plants, viz., seeds, tubers and roots, together with the highly specialized muscle tissue of animals, a wide variety of selection could form a suitable source of nutriment. In many of the studies in this series we observed deformities suggestive of rickets. Diets composed of cereals, legume seeds and muscle meats were especially likely to induce rickets-like deformities. We came to appreciate that such food mixtures were decidedly deficient in calcium and in fat-soluble A, and to a somewhat lesser degree in phosphorus. When we undertook a systematic analysis of the nature of the factors which operated to influence the skeletal changes observed, we judged it wise first to make the analysis of those food mixtures with which we had much experience, and accordingly in the first paper of this series is described the production of rickets-like conditions by diets of a simpler character. We have continued, however, in many instances, to restrict our animals to rather complex food mixtures such as are described in this paper. The several individual factors in each of these more complex food mixtures we are now able to evaluate with considerable accuracy, and the food mixtures serve our purpose well, notwithstanding the fact that at first glance the formulas may appear unnecessarily complex and confusing. In reality they can be regarded as nearly as simple as if derived from purified foodstuffs supplemented with small additions of articles especially rich in one or another of the uncharacterized dietary essentials.

The diets employed in securing the data discussed in this paper were all fairly satisfactorily constituted with respect to all dietary factors except calcium. When casein or steak was added, the mixture was improved both with respect to protein and phosphorus. Cereal and legume seed mixtures, no matter how complex, never furnish protein of the optimal quality, and their phosphorus content is somewhat below the plane at which nutrition is best promoted. Such mixtures fall far short of meeting the need of the body for calcium during growth, and, no matter how well they may be supplemented, malnutrition will result from specific starvation for calcium unless a supplementary source of supply of it is made available. As important as phosphate additions, but far less important than additions of calcium in such diets, is an abundant supply of fat-soluble A. Diets similarly constituted, but derived in great measure from degerminated cereal products, will be much more faulty with respect to each of these factors than when entire seed kernels are used.

The animals used in these experiments were confined for the periods indicated by the accompanying charts to diets in which the main defect was deficiency in calcium. In order to avoid confusion from the possible effects of variables in the diet of a minor character, such as small differences in the quality of the protein or in the quantity of phosphorus or of fat-soluble A, we prepared the diets in such manner that these three nutrient essentials were made more abundant than in other experiments. If in such diets as we are discussing sufficient calcium is added, this will improve their biological value to an extent which can be detected in increased vitality as shown by span of life, fertility and diminished mortality among the young. Since the acidity of the seed or seed and steak mixture might affect in some way the growth of the osseous tissue, we neutralized this in some diets by the inclusion of an appropriate amount of sodium bicarbonate.

The following diets were employed:

No. 2684.

	Per cent.
Wheat	30.0
Maize	20.0
Polished Rice	12.0
Rolled oats	11.5

Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	2.0

Ration 2684 is characterized by a marked deficiency in calcium. Since the ash of seeds is distinctly acid, ranging from the equivalent of 2.63 c.c. to 68.34 c.c. N/1 solution per 100 grams, we added in this case 1.5 gms. of sodium bicarbonate per 100 gms. of ration. The diet was thus made to contain a slight excess of bases over acids. For lack of calcium very little growth took place. This same food mixture with the sodium bicarbonate replaced by calcium carbonate induced optimal growth in the first generation, but, apparently owing to a deficiency of the food in phosphorus the later generations became undersized and the mortality among the young was high. On the diet of Lot 2684 the animals aged very early.

No. 2810.

	Per cent.
Whole wheat kernel	30.0
Whole maize kernel	14.5
Polished rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak (beef)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

Ration 2810 was closely similar to ration 2684, except that it contained 10 per cent. of round steak. This addition improved the quality of the seed proteins in a degree which can easily be demonstrated experimentally. It also raised the phosphorus content from .3047 to .3726 gm. per 100 gms. of mixture, at the same time increasing the acidity of the diet, because of the mineral content of the cereal and legume mixture, from the equivalent of 16.61 c.c. N/1 base solution to 5.75 c.c. N/1 acid solution per 100 gms. of the ration as fed. These rats were, therefore, on the same deficient intake of calcium as were lot 2684, but had much more phosphorus and a more acid food supply. The content of the fat-soluble A in their diet was also higher than that of Lot 2684 (Chart 1), but the latter were supplied with enough of this factor to fully meet the needs of the growing rat when all other dietary factors are satisfactory.

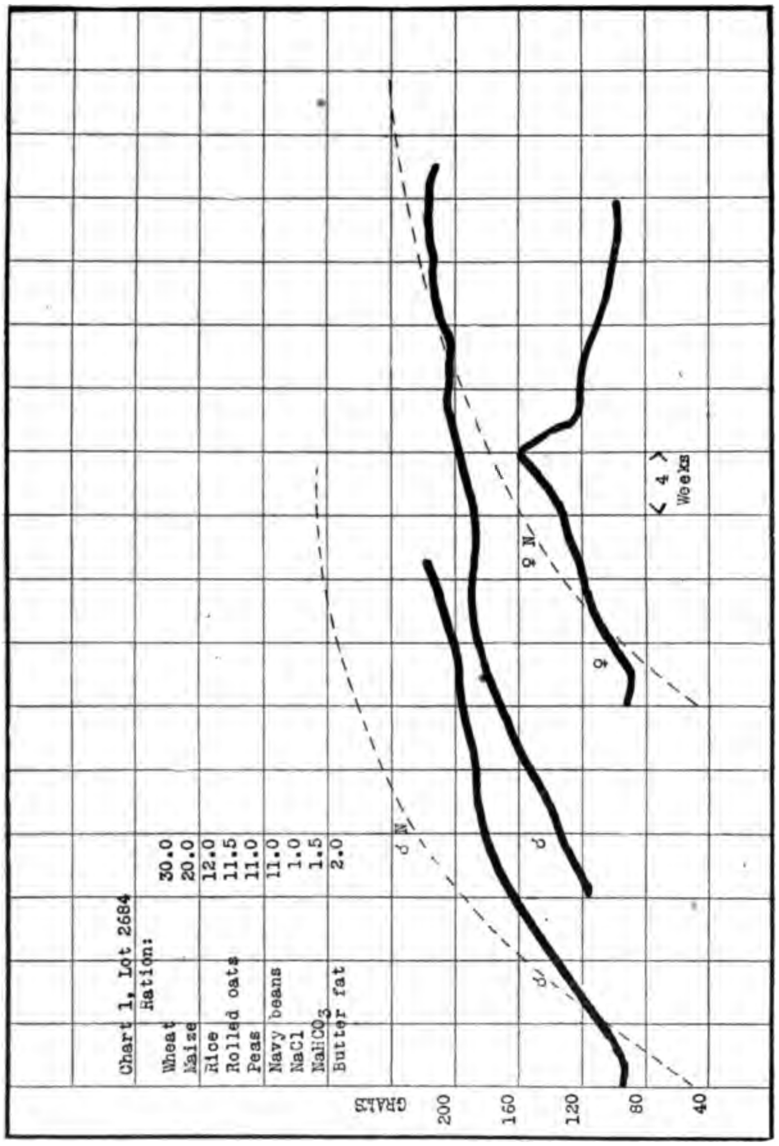
Chart 1, Lot 2684
Ration:
Wheat 30.0
Maize 20.0
Rice 12.0
Rolled oats 11.5
Peas 11.0
Navy beans 11.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 2.0
GRAMS
200
160
120
80
40
♂ N
♀ N
♂
♂
♀
< 4 >
Weeks

Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	2.0

Ration 2684 is characterized by a marked deficiency in calcium. Since the ash of seeds is distinctly acid, ranging from the equivalent of 2.63 c.c. to 68.34 c.c. N/1 solution per 100 grams, we added in this case 1.5 gms. of sodium bicarbonate per 100 gms. of ration. The diet was thus made to contain a slight excess of bases over acids. For lack of calcium very little growth took place. This same food mixture with the sodium bicarbonate replaced by calcium carbonate induced optimal growth in the first generation, but, apparently owing to a deficiency of the food in phosphorus the later generations became undersized and the mortality among the young was high. On the diet of Lot 2684 the animals aged very early.

No. 2810.

	Per cent.
Whole wheat kernel	30.0
Whole maize kernel	14.5
Polished rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Round steak (beef)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

Ration 2810 was closely similar to ration 2684, except that it contained 10 per cent. of round steak. This addition improved the quality of the seed proteins in a degree which can easily be demonstrated experimentally. It also raised the phosphorus content from .3047 to .3726 gm. per 100 gms. of mixture, at the same time increasing the acidity of the diet, because of the mineral content of the cereal and legume mixture, from the equivalent of 16.61 c.c. N/1 base solution to 5.75 c.c. N/1 acid solution per 100 gms. of the ration as fed. These rats were, therefore, on the same deficient intake of calcium as were lot 2684, but had much more phosphorus and a more acid food supply. The content of the fat-soluble A in their diet was also higher than that of Lot 2684 (Chart 1), but the latter were supplied with enough of this factor to fully meet the needs of the growing rat when all other dietary factors are satisfactory.

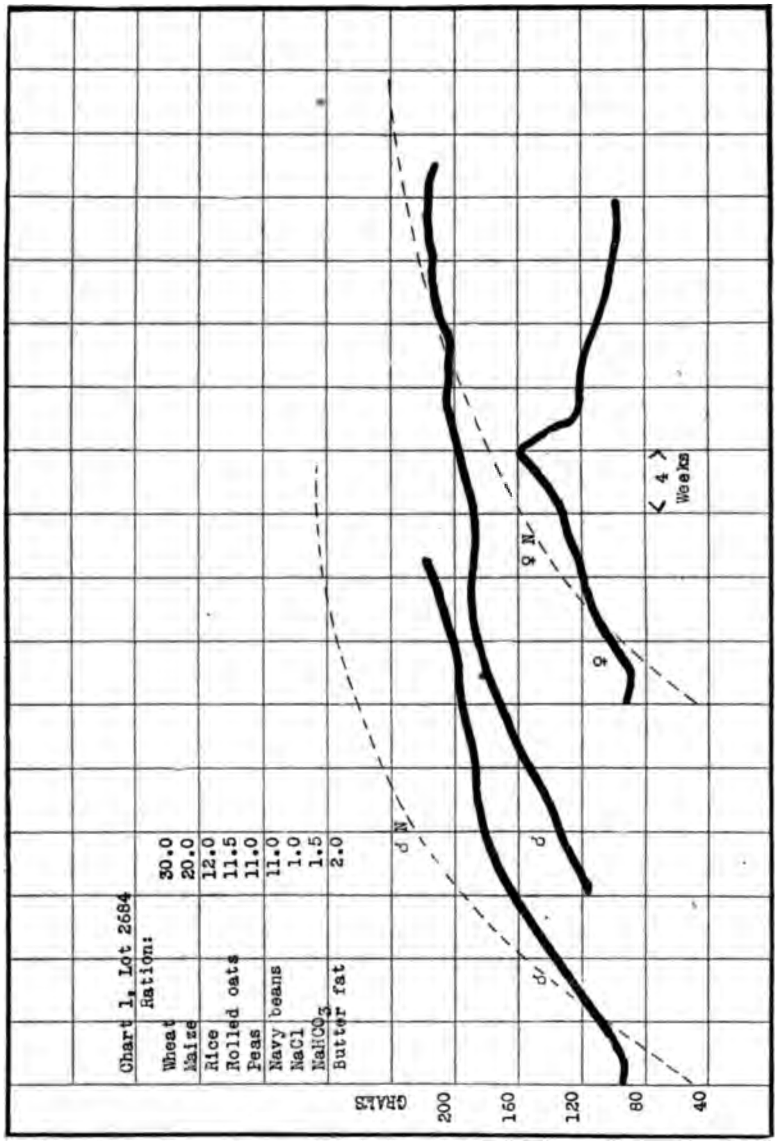
Chart 1, Lot 2684
Ration:
Wheat 30.0
Maize 20.0
Rice 12.0
Rolled oats 11.5
Peas 11.0
Navy beans 11.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 2.0
GRAMS
200
160
120
80
40
♂ N
♂
♂
♀ N
♀
< 4 >
Weeks

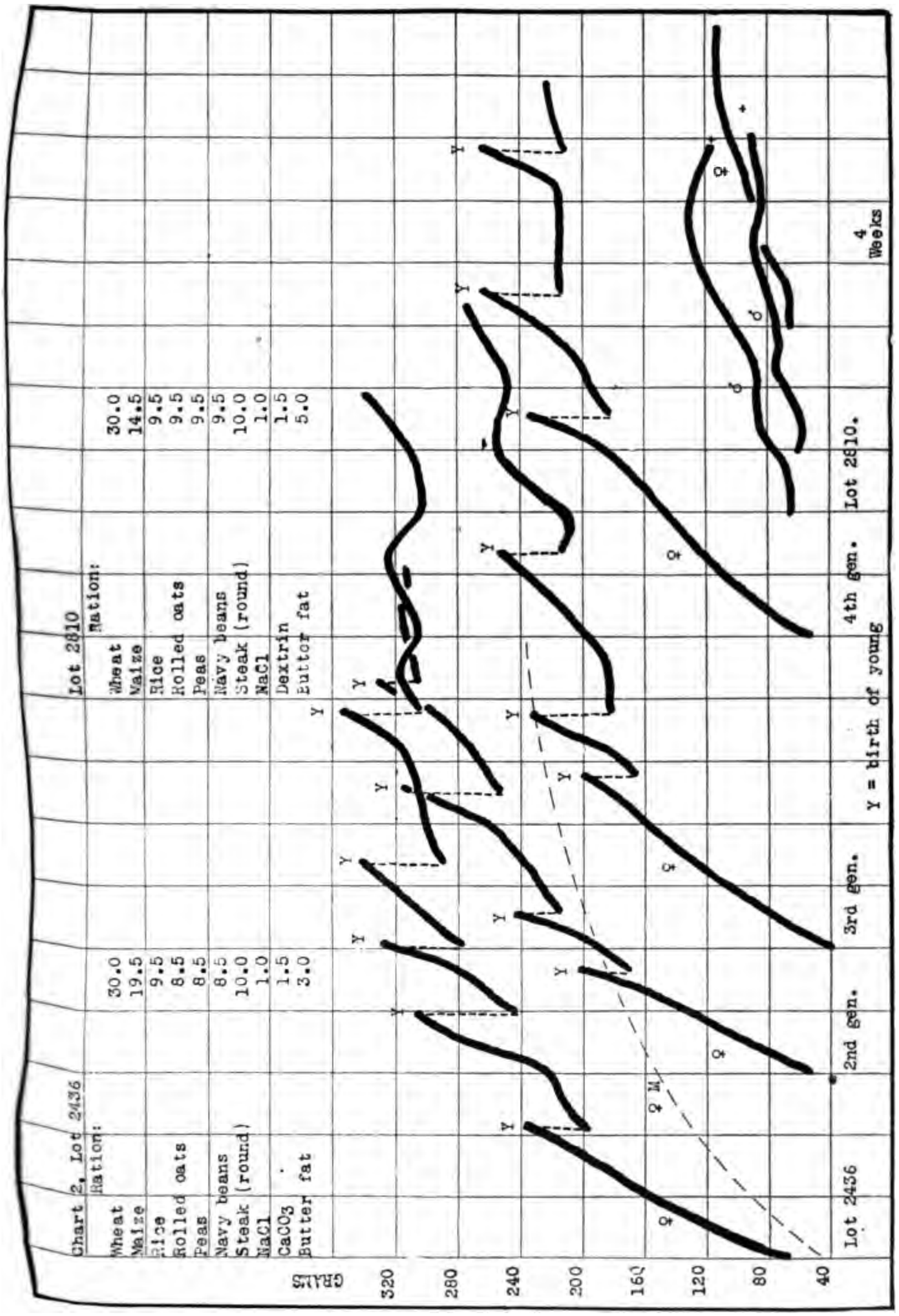
Chart 2, Lot 2436
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
CaCO3 1.5
Butter fat 3.0
Lot 2810
Ration:
Wheat 30.0
Maize 14.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Steak (round) 10.0
NaCl 1.0
Dextrin 1.5
Butter fat 5.0
GRAMS
320
280
240
200
160
120
80
40
Y
♀
♀ M
♂
Lot 2436
2nd gen.
3rd gen.
4th gen.
Lot 2810.
4 Weeks
Y = birth of young

Ration 2435 differed in its properties from ration 2731 in three respects. The former had a lower protein content and was poorer in phosphorus, because it lacked the beefsteak which the latter contained. Furthermore, it contained 1.5 per cent. of calcium carbonate instead of sodium bicarbonate. These modifications made a remarkable difference in the growth and vitality of the animals. Lot 2435 grew normally and showed high fertility, but the infant mortality was high. They deteriorated from generation to generation, because of the shortage of phosphorus, and the relatively poor quality of proteins. This group should be compared with Lot 2436 (Chart 2).

No. 2581.

	Per cent.
Beef liver (steamed and dried)	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	3.0

Ration 2581 was of good quality with respect to all constituents other than calcium, in which it was very deficient. The acidity of this food, owing to the high content of phosphorus and sulphur derived from liver and casein, was very great, and aside from the lack of calcium was the only known fault. It is of great interest from the standpoint of the establishment of the validity of our method of interpretation of the causes of the bone lesions, discussed in these experiments, to compare the histological picture seen in animals restricted to diets having the same faults, but derived chiefly from such widely different sources as cereals and legume seeds in one instance, and liver, casein, dextrin, butter fat and salts in the other. The effects on the bones were very similar.

On the diet of Lot 2581 all rats died very early. They were short, stunted, thin animals, with serious deformities of the thoraces.

This diet modified by the addition of calcium carbonate to the extent of 1.5 per cent., and without the addition of butter fat, induced approximately normal growth and fertility. There was no evidence of rachitic deformities. All the fat-soluble A and a specific antirachitic substance, if there is such a substance, were derived entirely from 25 per cent. of liver in the diet.

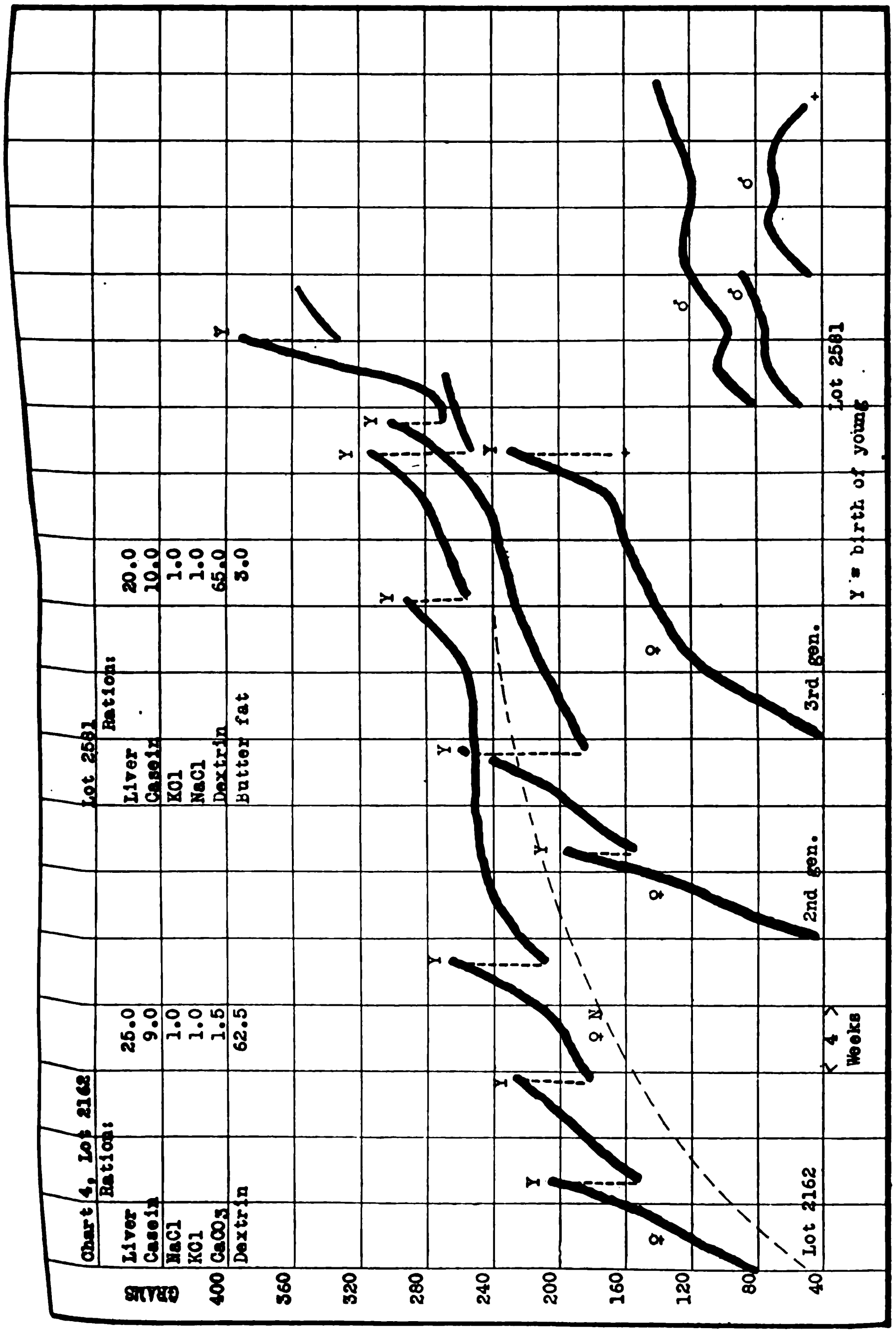
Chart 4, Lot 2162
Ration:
Liver 25.0
Casein 9.0
NaCl 1.0
KCl 1.0
CaCO₃ 1.5
Dextrin 62.5
Lot 2581
Ration:
Liver 20.0
Casein 10.0
KCl 1.0
NaCl 1.0
Dextrin 65.0
Butter fat 3.0
GRAMS
400
360
320
280
240
200
160
120
80
40
Y
♀
♀ N
2nd gen.
3rd gen.
♂
♂
♂
Lot 2162
Lot 2581
4 Weeks
Y = birth of young

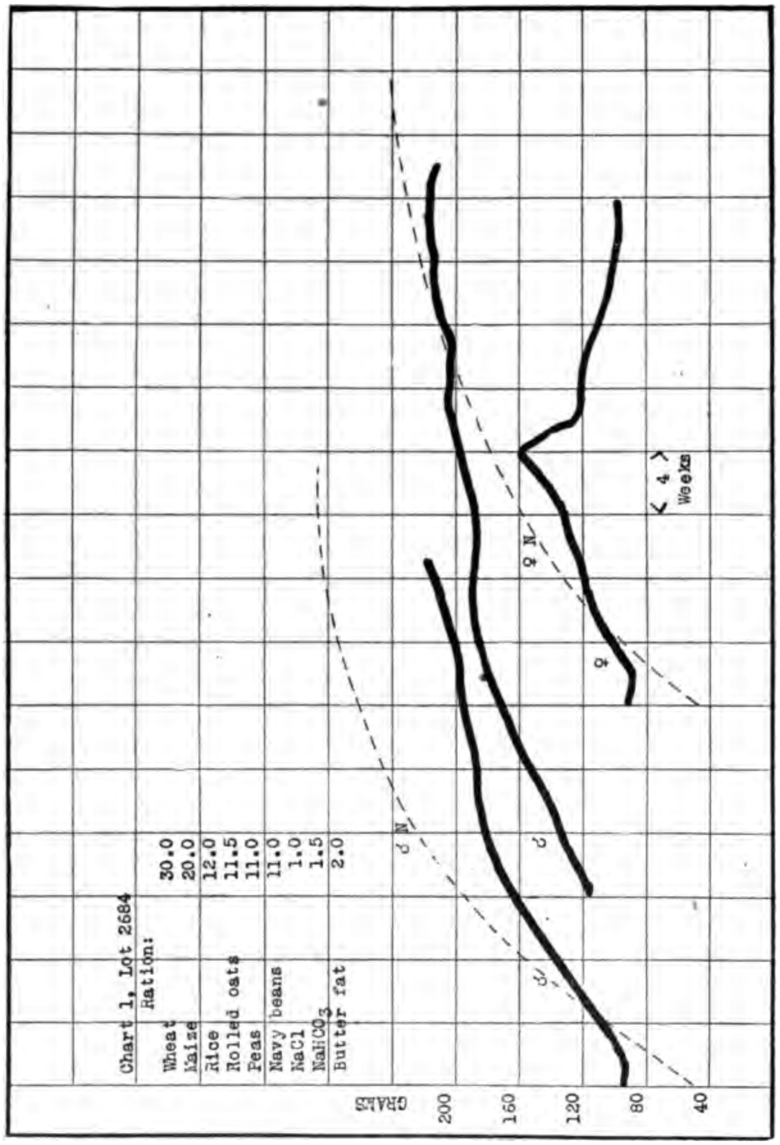

Chart 1, Lot 2584

Ration:

Wheat	30.0
Maize	20.0
Rice	12.0
Rolled oats	11.5
Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	2.0

No. 2436. (Control Experimental Ration.)

	Per cent.
Wheat	30.0
Maize	19.5
Polished Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Beefsteak	10.0
NaCl	1.0
$CaCO_3$	1.5
Butter fat	3.0

Ration 2436 was like ration 2810, except that the dextrin of the latter was replaced by 1.5 per cent. of calcium carbonate. This addition had a most remarkable effect in improving the quality of the diet. The animal appeared to be in the optimal condition and a family restricted to this diet appeared in the fourth generation to be entirely normal. One female of the fourth generation successfully weaned three litters (17 young).

No. 2731.

	Per cent.
Wheat	30.0
Maize	19.5
Polished Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	3.0

Ration 2731 was almost identical with ration 2810. It differed in containing 1.5 per cent. of sodium bicarbonate, which changed it from a distinctly acid to a slightly alkaline reaction. The animals placed upon it became short and stocky, were rough looking and died early.

No. 2435. (Control Experimental Ration.)

	Per cent.
Wheat	30.0
Maize	20.0
Polished Rice	11.0
Rolled oats	11.5
Peas	11.0
Navy beans	11.0
NaCl	1.0
$CaCO_3$	1.5
Butter fat	3.0

65757A

Ration 2435 differed in its properties from ration 2731 in three respects. The former had a lower protein content and was poorer in phosphorus, because it lacked the beefsteak which the latter contained. Furthermore, it contained 1.5 per cent. of calcium carbonate instead of sodium bicarbonate. These modifications made a remarkable difference in the growth and vitality of the animals. Lot 2435 grew normally and showed high fertility, but the infant mortality was high. They deteriorated from generation to generation, because of the shortage of phosphorus, and the relatively poor quality of proteins. This group should be compared with Lot 2436 (Chart 2).

No. 2581.

	Per cent.
Beef liver (steamed and dried)	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
Dextrin	65.0
Butter fat	3.0

Ration 2581 was of good quality with respect to all constituents other than calcium, in which it was very deficient. The acidity of this food, owing to the high content of phosphorus and sulphur derived from liver and casein, was very great, and aside from the lack of calcium was the only known fault. It is of great interest from the standpoint of the establishment of the validity of our method of interpretation of the causes of the bone lesions, discussed in these experiments, to compare the histological picture seen in animals restricted to diets having the same faults, but derived chiefly from such widely different sources as cereals and legume seeds in one instance, and liver, casein, dextrin, butter fat and salts in the other. The effects on the bones were very similar.

On the diet of Lot 2581 all rats died very early. They were short, stunted, thin animals, with serious deformities of the thoraces.

This diet modified by the addition of calcium carbonate to the extent of 1.5 per cent., and without the addition of butter fat, induced approximately normal growth and fertility. There was no evidence of rachitic deformities. All the fat-soluble A and a specific antirachitic substance, if there is such a substance, were derived entirely from 25 per cent. of liver in the diet.

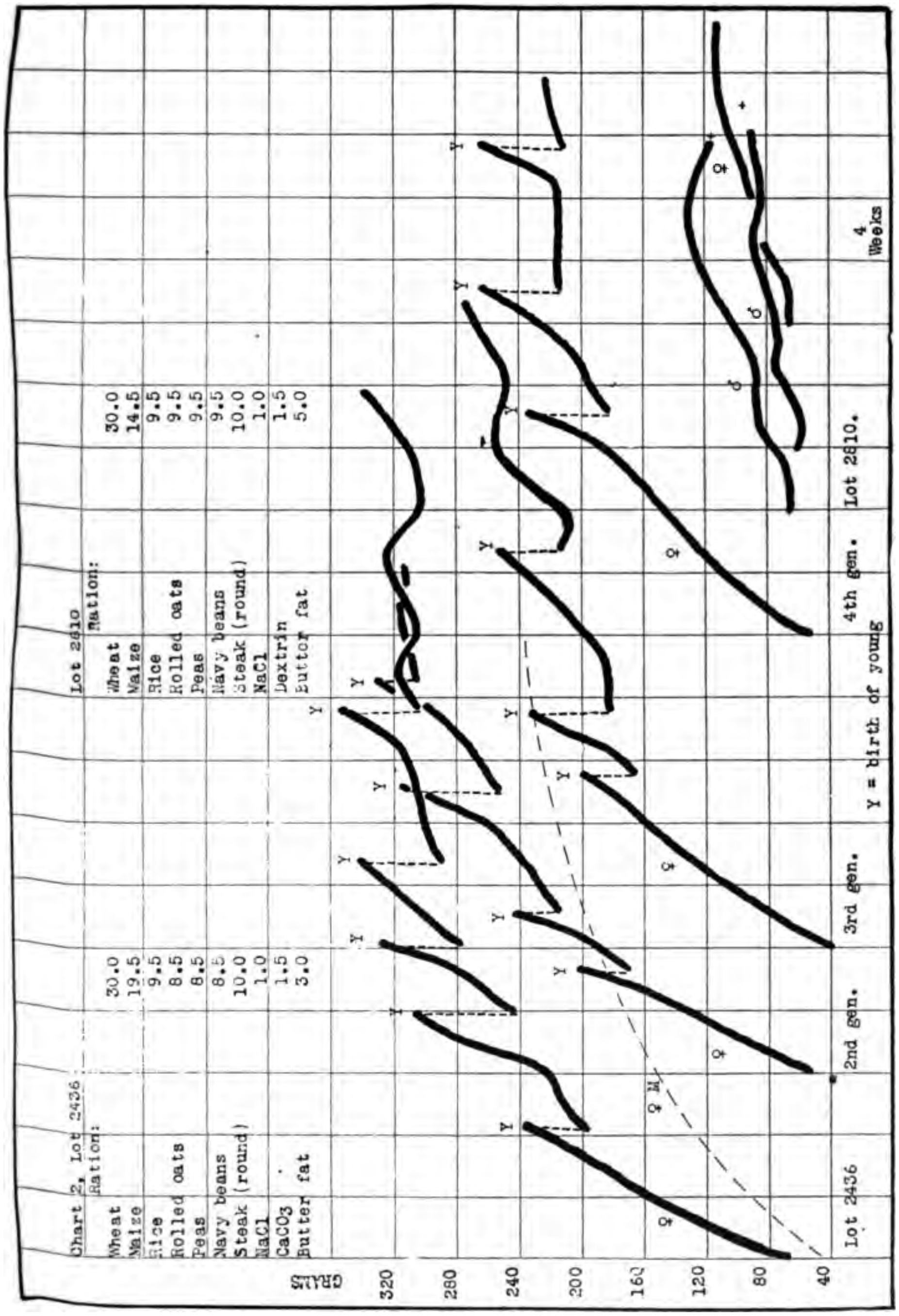

Chart 2, Lot 2436

Ration:	
Wheat	30.0
Maize	19.5
Rice	9.5
Rolled oats	8.5
Peas	8.5
Navy beans	8.5
Steak (round)	10.0
NaCl	1.0
$CaCO_3$	1.5
Butter fat	3.0

Lot 2810

Ration:	
Wheat	30.0
Maize	14.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Steak (round)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

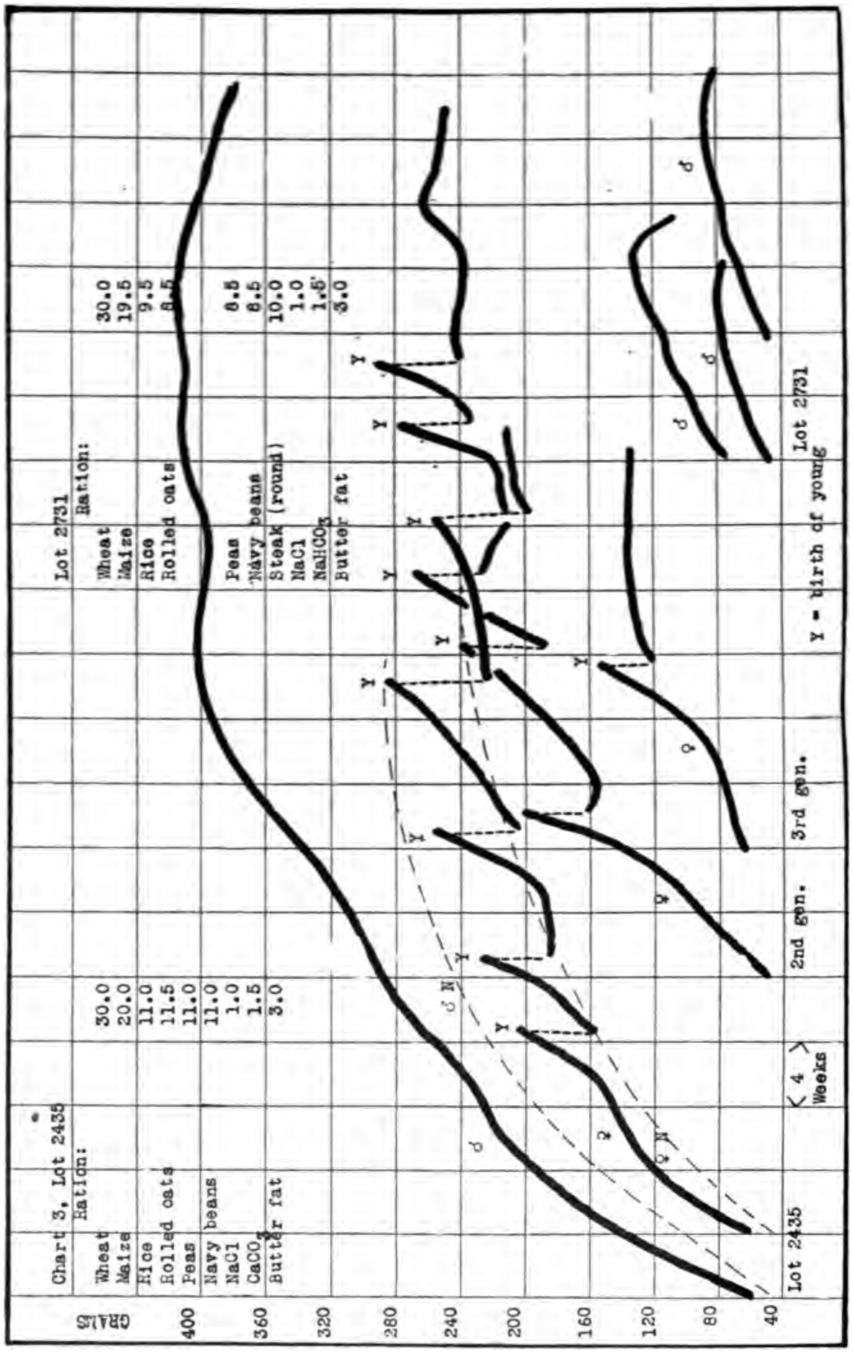
Chart 3, Lot 2435
Ration:
Wheat 30.0
Maize 20.0
Rice 11.0
Rolled oats 11.5
Peas 11.0
Navy beans 11.0
NaCl 1.0
$CaCO_3$ 1.5
Butter fat 3.0
Lot 2731
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 3.0
GRAMS
400
360
320
280
240
200
160
120
80
40
♂
♀
♂ N
♀ N
Y
Lot 2435
< 4 > Weeks
2nd gen.
3rd gen.
Lot 2731
Y = birth of young

junctions. When the thorax was opened, the junctions of the ribs with their cartilages were enlarged and there was marked angular deformity (Fig. 1). This extreme degree of angular deformity at the junctions of the ribs and the cartilages exceeded that found in the ribs of rats on a diet already described, low in fat-soluble A and phosphorus (4). The thymus was atrophied. The spleen was frequently greatly enlarged. There was little, if any, subcutaneous or visceral fat. The gonads were atrophic. The long bones cut easily. There were, as a rule, no fractures to be found in any of the bones except the ribs, which were usually studded throughout their length with the calluses of multiple healing fractures.

Microscopic examination of the bones revealed a lesion which was striking in its uniformity. The band of cartilage (Fig. 2) which separates the epiphyseal nucleus of ossification from the shaft was much broader than in a normal bone. The cells of the proliferative zone were separated one from another by very little matrix, and the cell bodies were almost in contact. Although there was a good deal of invasion of the cartilage by blood vessels from the shaft, the columnar arrangement of the cells was well maintained throughout. Because of the increased size and the invasion of the cartilage the cell columns seemed to radiate from the lower margin of the epiphyseal nucleus like the spokes of a wheel (Fig. 3). There was, as a rule, no calcification of the proliferative zone, though occasionally a few scattered patches of calcified matrix were present to represent a provisional zone of calcification. These calcified areas were usually at the periphery of the bone. The vascular sprouts which invaded the cartilage were very fine capillary vessels and were single or multiple. Often the invasion was carried on to a considerable distance from the shaft. Occasionally the invading vessels were arranged in tufts and destroyed wide areas of cartilage before them. The invasion of the cartilage usually took place, however, along the strands of intracellular matrix (Fig. 4). Cartilage which was directly in contact with the osteoid tissue seemed to have been protected against invasion by capillaries from the shaft. Frequently large sinuses, which had thin walls, made up of endothelium and of a few strands of connective tissue, were to be found just beneath the cartilage in the metaphysis (Fig. 5). At the junction of the cartilage with the metaphysis the cartilage cells had undergone changes like those which are described as degenerative in the bones of rachitic children. The cell cytoplasm had lost its affinity for basic dyes, and in specimens stained with haematoxylin

No. 2809.

	Per cent.
Liver	20.0
Casein	10.0
$NaHCO_3$	1.5
KCl	1.0
Dextrin	64.5
Butter fat	3.0

This ration was exactly similar to ration 2581, except that the sodium chloride was replaced by sodium bicarbonate. This neutralized the acidity of the former diet. That the reaction of the food within the limits of variation seen in this series of diets is not a factor of importance, the similarity in the character of the bone changes clearly show. There is no evidence that the neutralization of the acidity of the ration 2809 by the inclusion of sodium bicarbonate had any beneficial effect upon the animals. The gravity of the calcium deficiency was so great that the rats restricted to it were ruined by this deficiency alone. They developed deformities similar to those seen in the rats on ration 2581 (Chart 4).

No. 2143. (Control Experimental Ration.)

	Per cent.
Liver	20.0
NaCl	1.0
$CaCO_3$	1.5
KCl	1.0
Dextrin	73.5
Butter fat	3.0

Ration 2143 differed from ration 2809 in that it contained no casein, and all of its protein was derived from 20 per cent. of liver; and also in that it contained 1.5 per cent. of calcium carbonate. Even with the lower protein supply the animals were able, when a supply of calcium was available, to develop in a fairly satisfactory manner and to produce and rear some young. They showed no evidences of the deformities seen in Lot 2809.

PART II.

The earliest attempts to produce rickets in animals were made by feeding diets which were low in calcium. After the anatomical studies of Pommer had made clear that the fundamental lesions of the disease, in so far as the skeleton was concerned, were defects in the

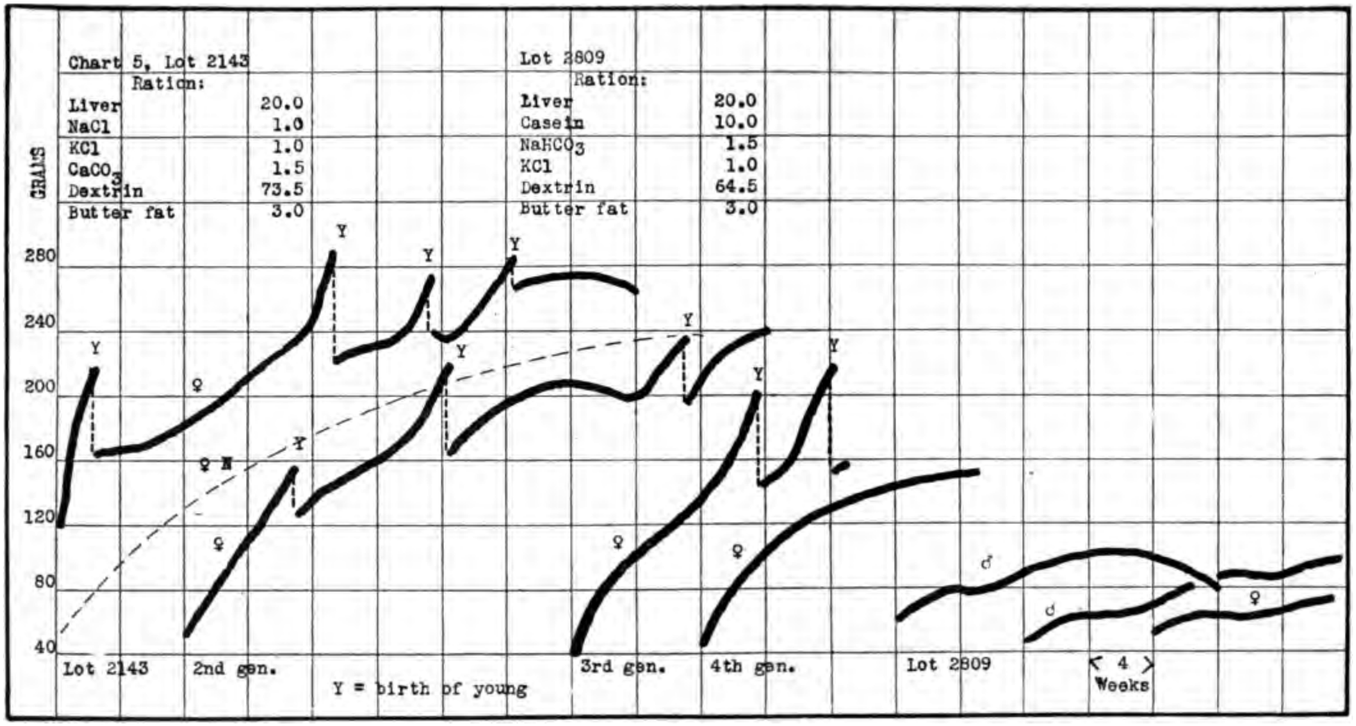
Chart 5, Lot 2143
Ration:
Liver 20.0
NaCl 1.0
KCl 1.0
CaCO3 1.5
Dextrin 73.5
Butter fat 3.0
Lot 2809
Ration:
Liver 20.0
Casein 10.0
NaHCO3 1.5
KCl 1.0
Dextrin 64.5
Butter fat 3.0
GRAMS
280
240
200
160
120
80
40
Y
♀
♀ II
♂
Lot 2143
2nd gen.
3rd gen.
4th gen.
Lot 2809
4
Weeks
Y = birth of young

calcification of cartilage and bone, it seemed logical to conclude that a deficiency in the intake of calcium might be the cause of the disease. Numbers of investigators have made attempts to produce rickets by feeding diets insufficient in calcium salts. At the present time, however, it is difficult to know what value to place upon their work. In the first place, it is almost impossible to decide whether the changes described in the bones of their animals were or were not equivalent to human rickets. In the second place, it is by no means easy to make certain that the deficiency in calcium in their diets was the sole fault. Almost all of the work of the investigators of this particular problem was performed prior to 1910. Before that time a diet which contained protein, carbohydrate and fat in sufficient amounts to furnish the energy requirements of the body, together with a supply of certain inorganic salts, was considered adequate for the maintenance of life and normal development over any number of generations. It stands to reason, therefore, that with the increased knowledge of today the recognition of dietary defects is easy in formulas which 15 years ago passed as deficient only as regards their content of calcium. A diet used by one of the investigators in question will suffice as an example. Dibbelt (1) maintained the dogs used in his experiments on a diet composed of horse meat, a carbohydrate (rice starch) and sodium chloride. This ration is low in calcium certainly, but it is also deficient in fat-soluble A, water-soluble B, and probably in the antiscorbutic substance as well. Moreover, the protein is not of the best quality. It will be seen that, even if there were no dispute as to whether the disease which was produced in puppies by this diet was rickets or not, it would be impossible to say that the deficiency in calcium was the cause.

The gross changes in the skeleton and the alterations in the finer structure of the bones of the rats placed on the various faulty rations described, which had in common a deficiency in calcium, were so similar that a single description will serve for all.

The bodies of the animals at autopsy were very much undersized and showed quite characteristic lesions. The coat was rough and the hair was irregular in length. The skulls were often very thin and easily compressible. The eyes were normal. The incisor teeth were very brittle, yellow and easy to extract. The epiphyses of the long bones, especially at the wrist and knee, were enlarged. The shafts of the bones were slender. The thoraces were much flattened from side to side, slender, and marked by deep grooves along the costochondral junctions. When the thorax was opened, the junctions of the ribs with their cartilages were enlarged and there was marked angular deformity (Fig. 1). This extreme degree of angular deformity at the junctions of the ribs and the cartilages exceeded that found in the ribs of rats on a diet already described, low in fat-soluble A and phosphorus (4). The thymus was atrophied. The spleen was frequently greatly enlarged. There was little, if any, subcutaneous or visceral fat. The gonads were atrophic. The long bones cut easily. There were, as a rule, no fractures to be found in any of the bones except the ribs, which were usually studded throughout their length with the calluses of multiple healing fractures.

Microscopic examination of the bones revealed a lesion which was striking in its uniformity. The band of cartilage (Fig. 2) which separates the epiphyseal nucleus of ossification from the shaft was much broader than in a normal bone. The cells of the proliferative zone were separated one from another by very little matrix, and the cell bodies were almost in contact. Although there was a good deal of invasion of the cartilage by blood vessels from the shaft, the columnar arrangement of the cells was well maintained throughout. Because of the increased size and the invasion of the cartilage the cell columns seemed to radiate from the lower margin of the epiphyseal nucleus like the spokes of a wheel (Fig. 3). There was, as a rule, no calcification of the proliferative zone, though occasionally a few scattered patches of calcified matrix were present to represent a provisional zone of calcification. These calcified areas were usually at the periphery of the bone. The vascular sprouts which invaded the cartilage were very fine capillary vessels and were single or multiple. Often the invasion was carried on to a considerable distance from the shaft. Occasionally the invading vessels were arranged in tufts and destroyed wide areas of cartilage before them. The invasion of the cartilage usually took place, however, along the strands of intracellular matrix (Fig. 4). Cartilage which was directly in contact with the osteoid tissue seemed to have been protected against invasion by capillaries from the shaft. Frequently large sinuses, which had thin walls, made up of endothelium and of a few strands of connective tissue, were to be found just beneath the cartilage in the metaphysis (Fig. 5). At the junction of the cartilage with the metaphysis the cartilage cells had undergone changes like those which are described as degenerative in the bones of rachitic children. The cell cytoplasm had lost its affinity for basic dyes, and in specimens stained with haematoxylin had a pale buff color which was indistinguishable from the staining reaction given by osteoid tissue. The nuclei of the cells were seen to be smaller and more pyknotic as the shaft was approached. Finally, the cartilage cell became buried in the osteoid tissue of the metaphysis, which it apparently helped to form. In other words, this so-called degeneration is in reality, as we pointed out in our description of the bones of rats on diets low in phosphorus and fat-soluble A, a metaplasia of the cartilage cells which results in their change into bone corpuscles.

The metaphysis in these bones was narrow, in contradistinction to the metaphysis in the bones of rats on a diet low in fat-soluble A and phosphate, and appeared to be in much greater degree a product of the diaphysis. The metaphysis was made up of large numbers of trabeculae of osteoid tissue, separated by blood vessels and a delicate reticular tissue containing a few elements from the bone marrow. The spongiosa (Fig. 5) was abundant, and the trabeculae of which it was composed were very numerous and slender. This thin character of the trabeculae was another point of difference between the bones of the rats on the diets low in calcium and those of rats fed on diets low in fat-soluble A and phosphorus. The trabeculae in the latter animals were much thicker, and were fewer in number. The osteoid was broad and often lamellar (Fig. 7). The borders of the osteoid tissue were sharp and clear cut; the cells were widely separated, and their nuclei were small and stained deeply. The osteoblasts which surrounded the trabeculae were flat, closely applied to the osteoid, and altogether resembled the endothelial cells of the blood vessels. In contrast, the osteoid tissue about the trabeculae of the rats on the diets deficient in calcium alone (Fig. 8) was much more ragged. The margins faded off into the surrounding tissue. The nuclei of the bone corpuscles were larger and were very close together, and the osteoblasts which surrounded the bone, instead of being flattened cells, were of low, columnar form, and were often piled up one on the other, to form two or three layers about the trabeculae. Occasionally trabeculae were seen in the rats on the diets deficient in calcium alone which showed osteoid tissue of both types. Calcification of the trabeculae in these animals was not as dense as in the normal bones. The deposits of lime salts were granular and the calcified zone usually merged gradually into the uncalcified border. The calcified cortex of the shaft was very thin, but the cortex as a whole was usually much thickened by a compensatory development of osteoid tissue (Fig. 9)

Frequently the cortex was canalized and was found in the form of a lattice work. In situations where the bone was under particular stress, e.g., at the sites of muscular attachment, or on the concave surface of the bone an enormous amount of osteoid tissue was formed between the periosteum and the original cortex (Fig. 9). This subperiosteal formation of osteoid tissue is analogous to the so-called "rachitic periostitis." It is in reality an hypertrophy to compensate for the diminished strength of the tissue used in building a bone. It is probable that canalization of the cortex of the bone and its growth in the form of a lattice has the same significance.

The bone marrow in the medullary cavity appeared normal in animals on these diets, but in the growing region of the bone the haematopoietic tissue was in a large measure replaced by delicate reticulum.

Many evidences of resorption of the trabeculae were present. Bones which showed evidences of resorption invariably contained numbers of large cells, the cytoplasm of which was filled with large basophil granules. These cells, which appeared to be derived, in part at least, from small, round lymphocyte-like forms, were arranged in a definite way along the borders of the trabeculae. They were found in the region of the bone which was being hollowed out to form a marrow cavity, but were not seen in the medulla itself. They were also present in large numbers in the interstices of the bony lattice work which represented the cortex. They were not present in the metaphysis to any extent, and were not found in the centers of ossification. We cannot say what their significance may be, but it would seem as though they must have some function to perform in connection with resorption.

Changes of the nature above described were present through the entire osseous system, but the pathological picture varied in severity in different bones. Those bones which grow most rapidly showed the most marked variations from the normal. The middle tier of ribs, the femur, the radius and ulna, and the tibia and fibula, suffered most severely. It was usual to find a more complete zone of provisional calcification in the cartilages of the vertebrae, phalanges and other smaller bones, even though the cartilages of the ribs and femur were without a vestige of lime salts. The osteoid borders of the trabeculae were widest where the most rapid growth was under way, for example, in the middle tier of ribs and the nuclei of ossification at the ends of the long bones. In the latter situation appositive bone formation must be very rapid, since in the process of growth the epiphyseal cartilage has to be replaced by bone during the period when the epiphysis as a whole is enlarging to keep pace with the growth of the entire body.

Broadly speaking, these bones had certain characteristics in common with the bones of rachitic children. The cartilages were not calcified, the epiphyseal line was irregular, and there was a considerable degree of invasion of the cartilage by blood vessels from the shaft. Osteoid tissue was formed in abnormal amounts, and the failure of calcification to occur resulted in weakness of the skeleton, with deformity of the thorax, cranio-tabes, etc. Attempted compensation for the lack of calcification of the bones was seen in the over-production of preosseous tissue and the subperiosteal hypertrophy of the cortex. On the other hand, the changes in the bones which were produced by the above described diets differed from rickets in several important particulars. The cartilage cells retained their columnar arrangement in spite of a considerable degree of invasion by blood vessels. Moreover, as Schmorl (7) has pointed out in the bones of puppies fed on low calcium diets, these bones showed evidences of a much greater degree of resorption than is usually seen in the bones of rachitic children.

If rats which had been confined for some weeks to one of the diets described above were given cod liver oil for a few days before they were killed, a healing process was induced which was evidenced by the formation of a provisional zone of calcification in the proliferative cartilage of the bones. If the animal was allowed to live for a sufficient length of time after the administration of cod liver oil was begun, the rachitic process in the bone was healed, and instead a condition of osteoporosis developed. If cod liver oil was included in the dietary formula from the beginning of its administration, the animals were never normal, and the bones at autopsy were absolutely different from those of rachitic rats. They showed a peculiar pathological condition which will be fully discussed in a subsequent paper.

When adequate amounts of calcium carbonate were added to the diets, the condition which developed in the skeleton was normal.

CONCLUSIONS.

1. Rats which have been fed on the diets described above, which are low in calcium, but which contain a sufficient supply of the fat-soluble A and have an approximately normal content of phosphate,

develop a pathological condition of the bone which has certain fundamental resemblances to human rickets. The condition is characterized by increased persistence of the epiphyseal cartilage, its invasion by blood vessels from the shaft, failure in lime salt deposition, the formation of a mixed zone between the cartilage and shaft (the rachitic metaphysis), and the over-production of osteoid tissue. Gross deformities which cannot be differentiated from those caused by rickets are seen at autopsy.

2. The above described pathological condition differs from rickets, as that disease commonly occurs in man, in that the arrangement of the proliferative zone of cartilage cells is maintained, and that the evidences of bone resorption in the diaphysis are excessive.

3. The rickets-like condition of the skeleton does not develop, if the deficiency in calcium is compensated for by the addition to the diet of calcium carbonate.

4. The rickets-like condition never develops, if the animal is allowed to receive cod liver oil, and the administration of cod liver oil, when the condition is well advanced, is followed by healing of the lesion with the formation of an osteoporotic bone.

5. Since this pathological condition in the skeleton is in no way prevented by an amount of butter fat which is amply sufficient to protect the animals against keratomalacia, it is necessary to assume that butter fat exercises a very feeble influence in protecting animals against the development of rickets-like lesions.

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EXPLANATION OF PLATES.

PLATES XXV.

FIG. 1. Longitudinal section through the cartilage and shaft of the tenth rib of a rat on a diet low in calcium, to show the extreme angulation at the costochondral junction. (Leitz micro-summar objective 135 mm.)

FIG. 2. Photomicrograph of a longitudinal section through the proximal end of the tibia of a rat on the diet of lot 2810. (Same magnification as in figure 1.)

PLATES XXVI.

FIG. 3. This photograph shows the invasion of the lower borders of the epiphyseal cartilage by capillary vessels from the diaphysis, and the radial arrangement of the columns of cartilage cells. Note the small amount of intercellular matrix and the close apposition of the cells. (Leitz objective No. 3.)

FIG. 4. Photograph with Leitz objective No. 6 showing a capillary blood vessel invading the epiphyseal cartilage. The vascular sprout is forcing its way along the septum which separates one cartilage cell from another.

PLATES XXVII.

FIG. 5. Large blood sinuses between the epiphyseal cartilage and the shaft of the bone. (Same magnification as figure 4.)

FIG. 6. Spongiosa of the shaft of the femur from a rat on diet of Lot 2581. Note the numerous, slender trabeculae, each surrounded by a zone of osteoid tissue. (Leitz No. 3 objective.)

PLATES XXVIII.

FIG. 7. High power photomicrograph of a trabecula with appositive osteoid. The cells in the osteoid are widely separated, the nuclei of the greater number are small. The osteoid border is wide and its edges are sharply cut. In the calcified portion of the trabecula is seen the cross section of a perforating blood vessel.

FIG. 8. High power photomicrograph (Leitz objective No. 6) to show the resorptive osteoid from the bone of an animal on a diet low in calcium, No. 2581. The osteoid border of these trabeculae is narrow. Calcification of the trabeculae is very poor. The trabeculae are crowded with large bone corpuscles, and at the border of the trabecula is seen a mass of osteoblasts four or five cells in depth. (O. S.)

PLATES XXIX.

FIG. 9. Longitudinal section through shaft of long bone to show subperiosteal-hyperplasia (Hyp.).

FIG. 10. Photomicrograph of callus about a spontaneous green stick fracture (f.). The (cal.), which is separated from the cortex by a mass of bone marrow, is almost entirely composed of osteoid tissue.

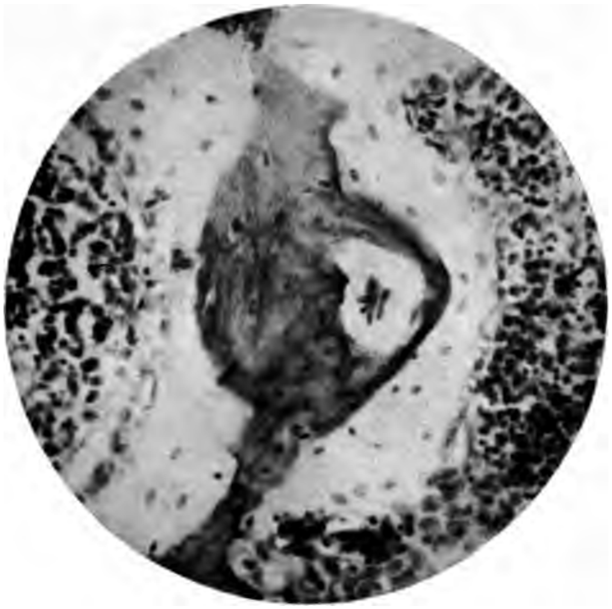


FIG. 7.

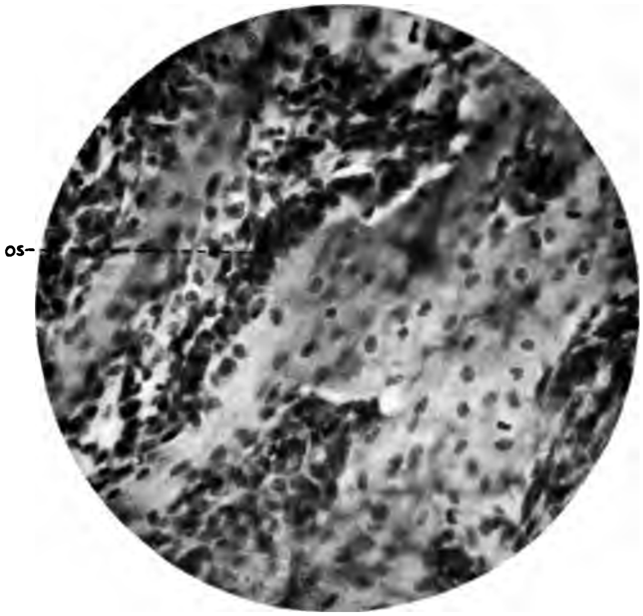
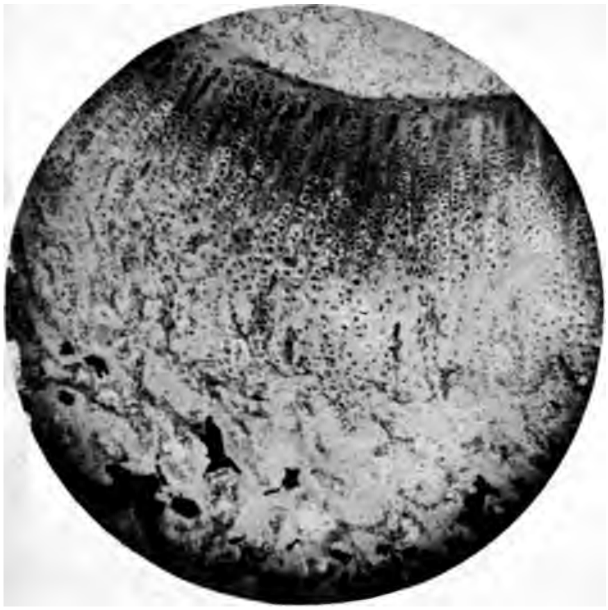


FIG. 8.

PLATE XXVIII.



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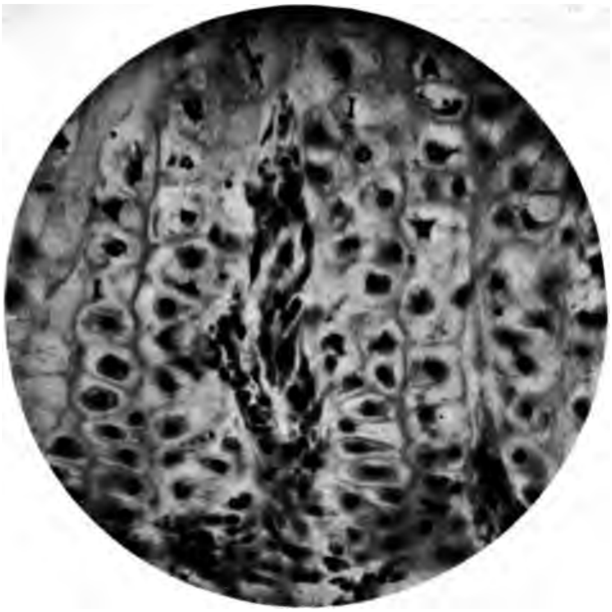

FIG. 4.

PLATE XXVI.

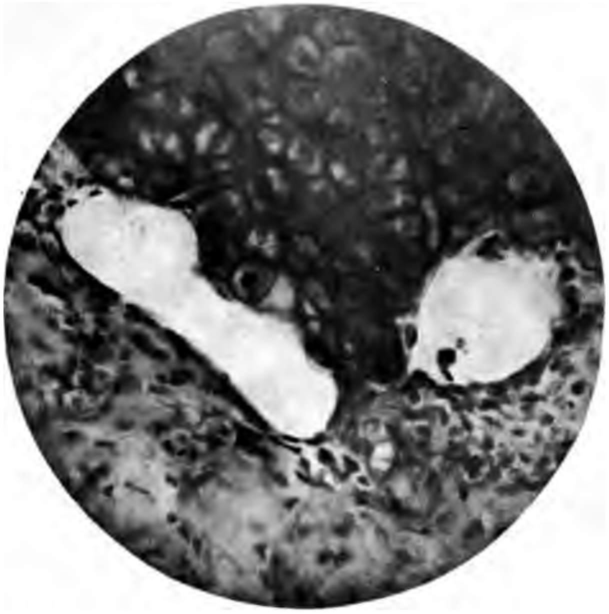

Fig. 5.

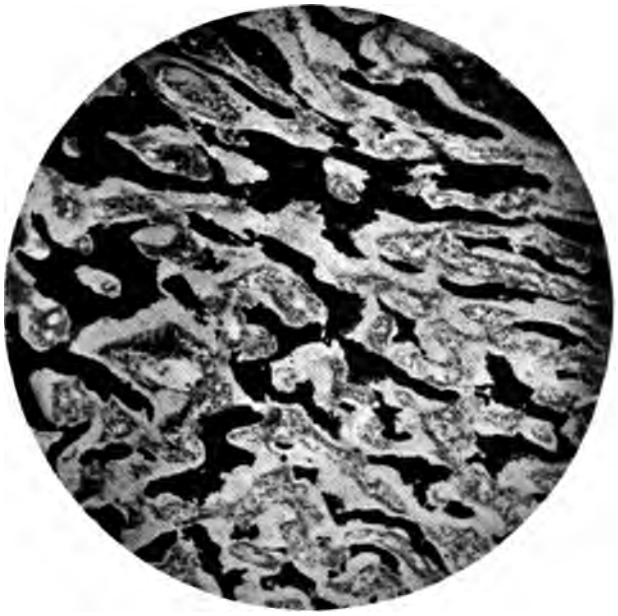

Fig. 6.

PLATE XXVII.

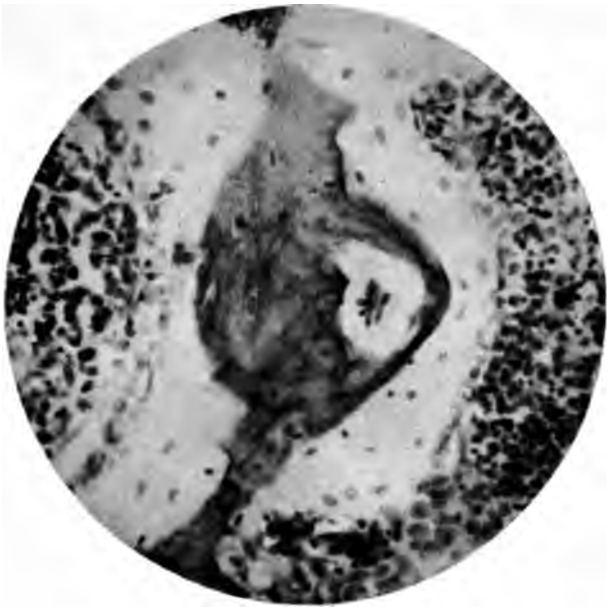

Fig. 7.

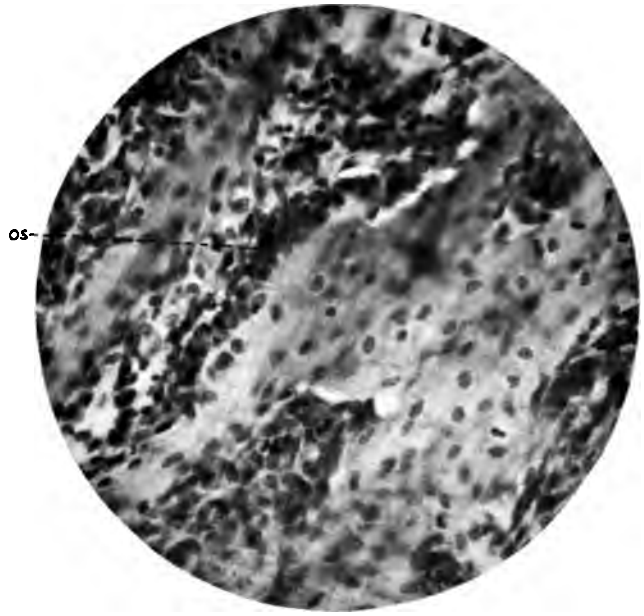

Fig. 8.

PLATE XXVIII.

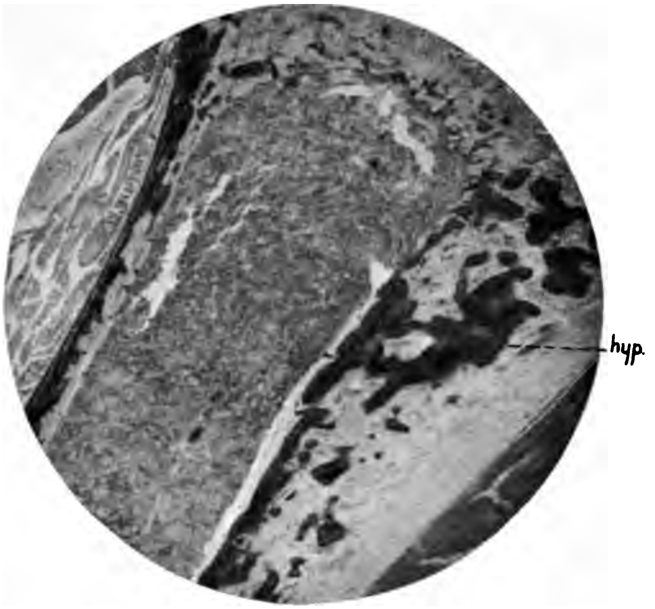

Fig. 9.

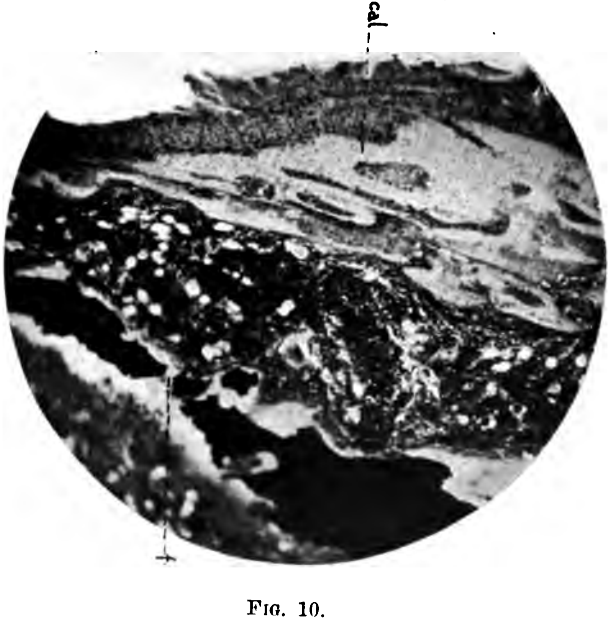

Fig. 10.

PLATE XXIX.

mic substance) does not in the absence of added calcium prove effective as an agent in the calcification of bone, but in diets markedly deficient in calcium with an approximately normal content of phosphorus, cod liver oil in surprising contrast to even much larger amounts of butter fat serves to protect the animal against defective calcification of the osseous tissue. The bones of rats which have been raised on a diet in which butter fat supplies the only source of the fat-soluble organic factor show a severe rickets-like condition which we have described at length elsewhere. These bones contain much osteoid tissue. The cartilages are not calcified and a pathological metaphysis is formed (Fig. 3). These animals show gross skeletal changes which can not be differentiated from those which are the result of rickets.

Rats of the second generation on such diets (Lot No. 2733) have bones which differ almost unbelievably from those of the parents. The cartilages are somewhat broader than normal, and very irregularly, or not at all calcified. There is very little invasion of the cartilage, however, and very little matrix between the cartilage cells. The bone itself is a mass of very slender trabeculae which may be fairly well calcified or may be composed almost entirely of osteoid tissue. In either event the trabeculae are filled with enormous bone corpuscles with large nuclei. Many of the bone corpuscles are surrounded by a clear area, although they are in touch with the surrounding bone through processes of their cytoplasm. The osteoblasts which surround the slender trabeculae are of enormous size and of low columnar or polyhedral form. Often there are several layers of them about a single trabecula. Sometimes the osteoblasts appear to be separated from the trabeculae which they surround, by capillary blood vessels. At least blood cells are to be found between the trabecula and the osteoblasts which surround it, though the vessel wall is usually not visible. In gross these animals are distinguished by the extreme slenderness of their bones. The skeleton is very fragile, fractures of the long bones are uncommon, but there are always multiple fractures of the ribs. More than 100 fractures have been present in a single thorax. The callus which is formed during the healing process is very scanty. Neither the epiphyses of the long bones nor the costochondral junctions are at all enlarged, and there is no gross deformity of the chest wall. We shall discuss the pathology of these animals and its significance in more detail in another communication.

We have, therefore, been fortunate in establishing experimental

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STUDIES ON EXPERIMENTAL RICKETS.

VII. THE RELATIVE EFFECTIVENESS OF COD LIVER OIL AS CONTRASTED WITH BUTTER FAT FOR PROTECTING THE BODY AGAINST INSUFFICIENT CALCIUM IN THE PRESENCE OF A NORMAL PHOSPHORUS SUPPLY.

BY P. G. SHIPLEY AND E. A. PARK

(From the Department of Pediatrics, Johns Hopkins University, Baltimore),

AND

E. V. McCOLLUM AND NINA SIMMONDS

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore).

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PLATES XXX TO XXXIII. (FIGURES 1 TO 6.)

In the first paper of this series* we pointed out that a diet which consisted of cereal grains, legume seeds, and muscle meat, supplemented with sodium chloride and sodium bicarbonate, was capable of supporting approximately normal growth, and the animals were capable of producing and rearing some young when 3 per cent. of cod liver oil was added to it. Essentially the same mixture, supplemented with 5 per cent. of butter fat, instead of the cod liver oil, failed to induce appreciable growth and led to permanent stunting, early deterioration and death. Our earlier studies showed that cereal, legume seed and muscle meat mixtures require additions of calcium, sodium, chlorine, and fat-soluble A, to render them sufficiently complete to make possible normal growth to maturity and approximately normal fertility.

The striking point about the experiments referred to is that the animals were so much more effectively protected against injury, due to the low calcium content of the diet by cod liver oil, than by butter fat, even when much more of the latter than of the former was added to the diet. This suggests that either (*a*) cod liver oil contains a dietary essential in abundance which is contained but sparingly in butter fat, or (*b*) that at least two organic nutritive principles are associated with certain fats, and that both butter fat and cod liver oil contain each, but in proportions widely different. With the following diet

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* Jour. Biol. Chem., 1921, xlv, 333.

	Per cent.
Rolled oats	30.0
Casein	18.0
Salts (185)	3.7
Agar-Agar	2.0
Dextrin	46.3

we have repeatedly demonstrated that xerophthalmia, but never rickets, develops. This we have discussed more extensively elsewhere.* The bones are *osteoporotic* but not *rachitic.* The inorganic constituents are present in a proportion which makes calcification complete, but some substance necessary for the formation of normal osseous tissue is lacking. Either butter fat or cod liver oil will furnish the necessary constituent in this particular diet.

With the following diet

No. 3041.

	Per cent.
Wheat	30.0
Corn	20.0
Peas	10.0
Navy beans	10.0
Rolled oats	10.0
Polished Rice	10.0
Casein	10.0

xerophthalmia never develops, but a pathological condition bearing fundamental resemblances to rickets invariably does. In this diet the faults are deficiency in calcium and low fat-soluble A. It should be kept in mind that in this diet fat-soluble A is not absent. Perhaps an estimate that it furnishes 50 per cent. of the optimal requirement of the growing rat for this substance would not be far from correct.

We have abundantly demonstrated, however, that the addition of butter fat alone to this diet will not cause optimal growth and skeletal development. It will, as a matter of fact, judging from the growth curves so far obtained, improve the condition of animals on this diet scarcely at all, even though the amount added may be anywhere between 3 and 20 per cent. of the food mixture. This observation we can now bring into contrast with the results of feeding a similar food mixture in which the deficiency was calcium alone, and on which the condition of the animals differed in the most pronounced degree, depending on whether the fat added to the diet was cod liver oil or butter fat.

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* Johns Hopkins Hospital Bulletin, May, 1921.

Calcium is a more serious limiting factor as regards growth in a seed and steak diet than is any organic substance. The presence of even liberal amounts of butter fat (ten to fifteen times the amount necessary to protect against ophthalmia on a diet of isolated food substances) proves to be little or no benefit in preventing injury due to insufficient calcium. Cod liver oil, however, is of much higher biological value for protecting rats against a lime salt deficiency. This protection is manifested in stimulation of growth and in greatly improved appearance, but although these rats may seem exceptionally well-nourished and appear to possess great vigor, they are actually in a state of nutritional instability. As a matter of fact, their vitality is far below that of rats on highly satisfactory diets. Their weakness, moreover, is shown in a spectacular way in its effect on the females as regards nursing their young. Such female rats show characteristic behavior, they are nervous and apprehensive, in walking they stand with legs more extended, and with the body held higher than is the case with a normal animal. Their gait is unsteady and slightly tottering. When at rest they have some difficulty in getting on their feet. Not infrequently the mothers die after nursing litters for 16–18 days, although they appear externally to be in good condition. This is especially to be emphasized in view of the fact that the animals on these low calcium diets which were enabled to grow fairly well by reason of the administration of cod liver oil were distinctly below the normal in their physical development.

They sometimes succeed in weaning their young, they frequently do not, especially with second or later litters. They only rarely destroy their young soon after birth, but are not able to nourish them satisfactorily with their milk. The difference between the protective value of butter fat and cod liver oil, however, is manifested in the difference of fertility of rats fed on one of these fats, as contrasted with the other. We have never been able to secure young with diets low in calcium of the type described in Chart 1. On the other hand, cod liver oil to the extent of 1 to 3 per cent. is very effective in promoting fertility. These young restricted to the mother's diet are short and stocky, rough-haired, with very poor skeletons, but little calcified, and die early.

Butter fat, although a good source of fat-soluble A (anti-ophthalmic substance) does not in the absence of added calcium prove effective as an agent in the calcification of bone, but in diets markedly deficient in calcium with an approximately normal content of phosphorus, cod liver oil in surprising contrast to even much larger amounts of butter fat serves to protect the animal against defective calcification of the osseous tissue. The bones of rats which have been raised on a diet in which butter fat supplies the only source of the fat-soluble organic factor show a severe rickets-like condition which we have described at length elsewhere. These bones contain much osteoid tissue. The cartilages are not calcified and a pathological metaphysis is formed (Fig. 3). These animals show gross skeletal changes which can not be differentiated from those which are the result of rickets.

Rats of the second generation on such diets (Lot No. 2733) have bones which differ almost unbelievably from those of the parents. The cartilages are somewhat broader than normal, and very irregularly, or not at all calcified. There is very little invasion of the cartilage, however, and very little matrix between the cartilage cells. The bone itself is a mass of very slender trabeculae which may be fairly well calcified or may be composed almost entirely of osteoid tissue. In either event the trabeculae are filled with enormous bone corpuscles with large nuclei. Many of the bone corpuscles are surrounded by a clear area, although they are in touch with the surrounding bone through processes of their cytoplasm. The osteoblasts which surround the slender trabeculae are of enormous size and of low columnar or polyhedral form. Often there are several layers of them about a single trabecula. Sometimes the osteoblasts appear to be separated from the trabeculae which they surround, by capillary blood vessels. At least blood cells are to be found between the trabecula and the osteoblasts which surround it, though the vessel wall is usually not visible. In gross these animals are distinguished by the extreme slenderness of their bones. The skeleton is very fragile, fractures of the long bones are uncommon, but there are always multiple fractures of the ribs. More than 100 fractures have been present in a single thorax. The callus which is formed during the healing process is very scanty. Neither the epiphyses of the long bones nor the costochondral junctions are at all enlarged, and there is no gross deformity of the chest wall. We shall discuss the pathology of these animals and its significance in more detail in another communication.

We have, therefore, been fortunate in establishing experimental

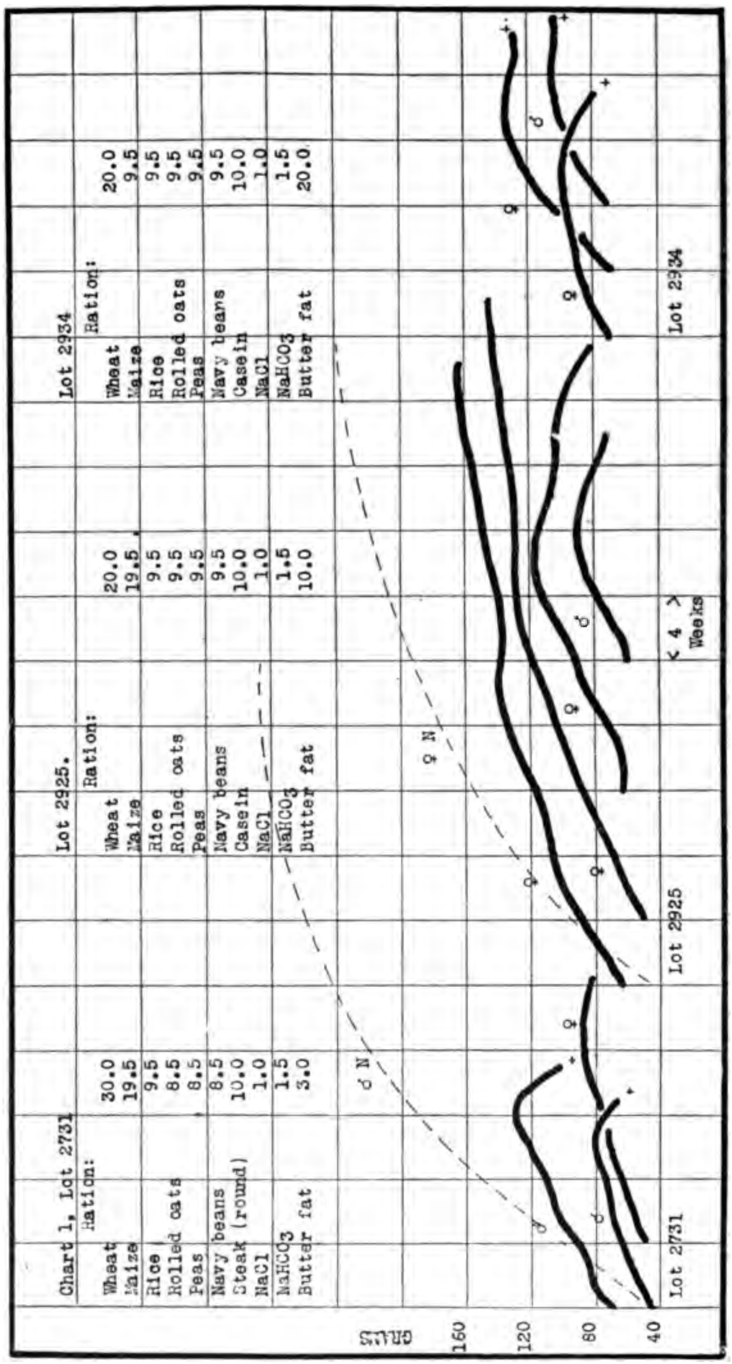
Chart 1, Lot 2731
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 8.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 3.0
Lot 2925.
Ration:
Wheat 20.0
Maize 19.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Casein 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 10.0
Lot 2934
Ration:
Wheat 20.0
Maize 9.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Casein 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 20.0
GRAMS
160
120
80
40
♂ N
♀ N
♂
♀
+
Lot 2731
Lot 2925
< 4 >
Weeks
Lot 2934

inorganic substances are contained in a food, bone formation can take place in a fairly normal manner, without the aid of the substance designated above as X. It is difficult to explain the observed results on any other assumption. It seems very suggestive, that as the content of calcium and of phosphate in the food deviates from the optimum ratio, either upward or downward and in either direction, there arises a need for a regulatory substance which aids in directing the osteoblast's activity toward the most favorable objective from the standpoint of the individual. Its purpose is to turn the bone corpuscle toward normality. The need for this hypothetical X becomes greater, the more decided the departure of the diet from the optimum in calcium and phosphorus ratios. When these components are present in optimal amounts the need for the regulatory factor certainly is very small as compared with what it is when either calcium or phosphate or both are furnished by the diet in abnormal amounts or proportions.

CHART 2. Lots 2732 and 2733 were restricted throughout the experiment to which were identical with that of Lot 2731 (Chart 1), except that cod liver replaced the butter fat. The remarkable benefit which the animals derived even such small amounts of cod liver oil as 1 to 3 per cent., as contrasted with even [illegible] or 20 per cent. of butter fat, is easily seen by comparing the curves of Chart 2 with those of Chart 1. The former not only grew much better, but looked well-nourished and produced and reared young to the weaning stage, whereas the animals whose records are shown in Chart 1 were rough-haired and poorly nourished, and were infertile.

Lot 2732 contained 4 females. Three of these had four litters (18 young). These were never normal, but the mothers reacted normally toward them and never destroyed them. The young remained under-sized and developed badly fractured ribs. Their abdomens were distended and they died early.

Lot 2733 had a similar history so far as reproduction was concerned.

CHART 3. Lot 2436 shows in a striking manner the effect of adding 1.5 per cent. of calcium carbonate to the diet of Lot 2731, Chart 1. These two groups of curves should be compared with Lots 2732 and 2733, Chart 2, and with Lots 2925 and 2934, Chart 1. The amount of calcium contained in a seed and steak mixture is very inadequate to meet the needs of the growing rat for this element. The small content of butter fat (3 per cent.) sufficed in this case, where the content of both calcium and phosphorus were near the optimum, to meet all the needs of the osseous system for any organic factor which is concerned with the functioning of the osteoblast. Yet in the absence of sufficient calcium in a nutritive situation otherwise identical, 20 per cent. of butter fat failed signally as compared with 1 or 3 per cent. of cod liver oil, to preserve the health and vitality of the animals or to enable them to grow. Such evidence may possibly suggest the supposition that cod liver oil contains a substance distinct from fat-soluble A and not hitherto recognized in working hypotheses relating to the interpretation of the causes of nutritive failure. In this event butter fat would have to contain traces of this substance, for when the calcium and phosphorus are present in favorable ratios and in near optimal concentrations, as in the diet of Lot 2436, butter fat furnishes everything necessary for so completing the seed-steak formula used as to make possible the formation of normal bones.

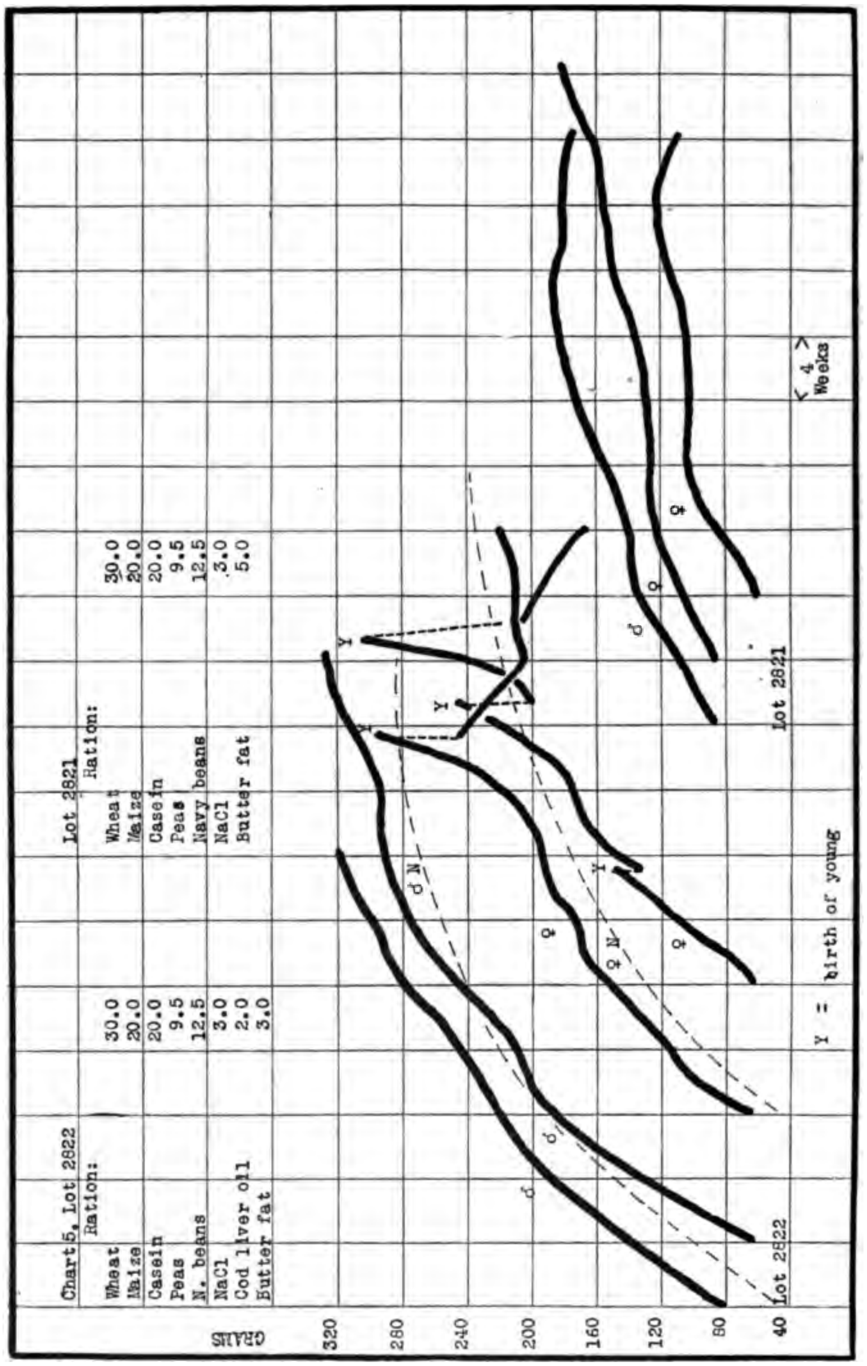
Chart 5, Lot 2822
Ration:
Wheat 30.0
Maize 20.0
Casein 20.0
Peas 9.5
N. beans 12.5
NaCl 3.0
Cod liver oil 2.0
Butter fat 3.0
Lot 2821
Ration:
Wheat 30.0
Maize 20.0
Casein 20.0
Peas 9.5
Navy beans 12.5
NaCl 3.0
Butter fat 5.0
GRAMS
320
280
240
200
160
120
80
40
♂
♂
♂ N
♀
♀ N
♀
Y
Y
Y
Y
♂
♀
♀
Lot 2822
Lot 2821
Y = birth of young
< 4 >
Weeks

conditions which clearly show that butter fat and cod liver oil not only contain the anti-ophthalmic substance, fat-soluble A, but that cod liver oil even in small amounts (2–3 per cent. of the diet) will, under the experimental conditions here described, protect against the baneful influence of serious grades of calcium starvation when other factors in the diet are satisfactory, whereas butter fat does not possess this power in a degree demonstrable by our method of study. It is probable that butter fat also contains minimal amounts of this organic factor, which is so abundant in cod liver oil, but the antirachitic effect of any samples of butter fat which we have employed is certainly very small as compared with the antixerophthalmic potency. This difference between the effects of cod liver oil, as contrasted with butter fat, can up to the present time be demonstrated only under the experimental conditions here described, viz., where there is a decided deficiency in the calcium content of the food.

In all the reproduction records which we have observed in these experiments, in which cod liver oil in the absence of sufficient calcium in the diet stimulated growth and improved the vitality of the animals so as to enable them to produce young, we have never seen a case where the mother destroyed her young even though she could not nourish them satisfactorily with her milk. In a recent discussion of the abnormal attitude which mother rats frequently take toward their young when restricted to diets in which the protein is inadequate, we called attention to the infanticidal tendency caused by this deficiency. It is interesting that this tendency to destroy the young is not caused by any fault in the diet which places the mother in a condition of nutritive instability in which she may easily break down while nursing a litter, or in which she fails in her attempt to induce normal nutrition in her offspring.

CONCLUSIONS.

1. Diets in which the calcium is deficient, but in which the phosphate content is near the optimum, cause injury to young rats, interfering with growth and causing characteristic disturbances in the growth and ossification of the skeleton.

2. Butter fat, even in liberal amounts, fails to be very effective in protecting the animals against this injury due to calcium deficiency.

3. Cod liver oil, on the other hand, is remarkably effective in promoting growth under the experimental conditions described, and in protecting young animals against the harmful effects of partial calcium starvation.

4. These results suggest the possibility that a dietary essential distinct from the anti-ophthalmic substance (fat-soluble A) may exist. If this is the case this would appear to be present in butter fat in small amounts, but to be very abundant in cod liver oil.

5. When the amount of calcium in the diet is below the optimum, but the phosphorus is normal, the rôle of the antirachitic organic substance is to direct as far as possible the bone development in the direction of normal.

6. When the calcium and phosphorus content of the diet, and presumably the other mineral elements, are nearly optimal, the amount of antirachitic substance needed by the body is small, and its function appears to be regulatory. The need for this substance is greatly increased when there is partial starvation for calcium.

7. We have not found it possible to demonstrate experimentally a difference between the effects of cod liver oil and butter fat when the content of the diet in calcium and phosphorus is near the optimum, the other elements in the diet being satisfactory.

EXPLANATION OF CHARTS.

CHART 1. Lots 2731, 2925 and 2934 illustrate the failure of butter fat, when added to the extent of 3 to 10 and 20 per cent. respectively, to stimulate growth in animals fed the diets described in the chart. The sole deficiency of appreciable magnitude in these food mixtures is calcium. This is shown by a comparison of the curves of these groups with those of Lot 2436, Chart 3. In the latter group the diet was identical with that of 2731, except that the sodium bicarbonate was replaced by calcium carbonate. The addition of calcium caused normal growth, high fertility and low infant mortality. Even a fourth generation female successfully reared young of the fifth generation on this diet, and there was no noticeable deterioration in the vitality of these descendants.

Lots 2731, 2925 and 2934 were in a "twilight zone" of nutritional instability. There appears to be no significance in the somewhat longer life span of some of Lot 2925 as compared with the other groups.

Even 3 per cent. or less of butter-fat contains sufficient fat-soluble A to prevent ophthalmia, and is sufficiently potent, so that when both calcium and phosphate are present in optimal concentrations at least fairly satisfactory bone growth is possible. Even 20 per cent. of butter fat does not, however, further appreciably promote their well-being under the conditions of those experiments where the calcium and phosphorus content of the diet are unsatisfactory.

The interpretation of experimental data such as we are describing, with a view to determining whether there are two uncharacterized dietary factors in such fats as butter fat or cod liver oil, in which hitherto only fat-soluble A was recognized, is rendered very difficult because of the impossibility up to the present time of separating the two, if there be two, and studying them independently. We are confronted with the question as to whether, when all indispensable organic and inorganic substances are contained in a food, bone formation can take place in a fairly normal manner, without the aid of the substance designated above as X. It is difficult to explain the observed results on any other assumption. It seems very suggestive, that as the content of calcium and of phosphate in the food deviates from the optimum ratio, either upward or downward and in either direction, there arises a need for a regulatory substance which aids in directing the osteoblastic activity toward the most favorable objective from the standpoint of the individual. Its purpose is to turn the bone corpuscle toward normality. The need for this hypothetical X becomes greater, the more decided the departure of the diet from the optimum in calcium and phosphorus ratios. When these components are present in optimal amounts the need for the regulatory factor certainly is very small as compared with what it is when either calcium or phosphate or both are furnished by the diet in abnormal amounts or proportions.

CHART 2. Lots 2732 and 2733 were restricted throughout the experiment to diets which were identical with that of Lot 2731 (Chart 1), except that cod liver oil replaced the butter fat. The remarkable benefit which the animals derived from even such small amounts of cod liver oil as 1 to 3 per cent., as contrasted with even 10 or 20 per cent. of butter fat, is easily seen by comparing the curves of Chart 2 with those of Chart 1. The former not only grew much better, but looked well-nourished and produced and reared young to the weaning stage, whereas the animals whose records are shown in Chart 1 were rough-haired and poorly nourished, and were infertile.

Lot 2732 contained 4 females. Three of these had four litters (18 young). These were never normal, but the mothers reacted normally toward them and never destroyed them. The young remained under-sized and developed badly fractured ribs. Their abdomens were distended and they died early.

Lot 2733 had a similar history so far as reproduction was concerned.

CHART 3. Lot 2436 shows in a striking manner the effect of adding 1.5 per cent. of calcium carbonate to the diet of Lot 2731, Chart 1. These two groups of curves should be compared with Lots 2732 and 2733, Chart 2, and with Lots 2925 and 2934, Chart 1. The amount of calcium contained in a seed and steak mixture is very inadequate to meet the needs of the growing rat for this element. The small content of butter fat (3 per cent.) sufficed in this case, where the content of both calcium and phosphorus were near the optimum, to meet all the needs of the osseous system for any organic factor which is concerned with the functioning of the osteoblast. Yet in the absence of sufficient calcium in a nutritive situation otherwise identical, 20 per cent. of butter fat failed signally as compared with 1 or 3 per cent. of cod liver oil, to preserve the health and vitality of the animals or to enable them to grow. Such evidence may possibly suggest the supposition that cod liver oil contains a substance distinct from fat-soluble A and not hitherto recognized in working hypotheses relating to the interpretation of the causes of nutritive failure. In this event butter fat would have to contain traces of this substance, for when the calcium and phosphorus are present in favorable ratios and in near optimal concentrations, as in the diet of Lot 2436, butter fat furnishes everything necessary for so completing the seed-steak formula used as to make possible the formation of normal bones.

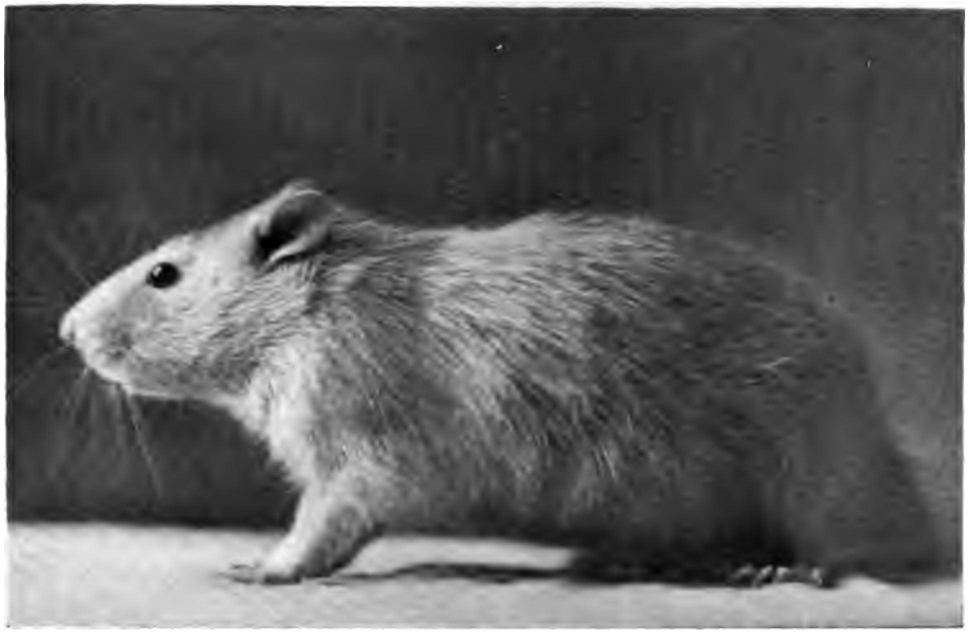

Fig. 2.

PLATE XXXI.

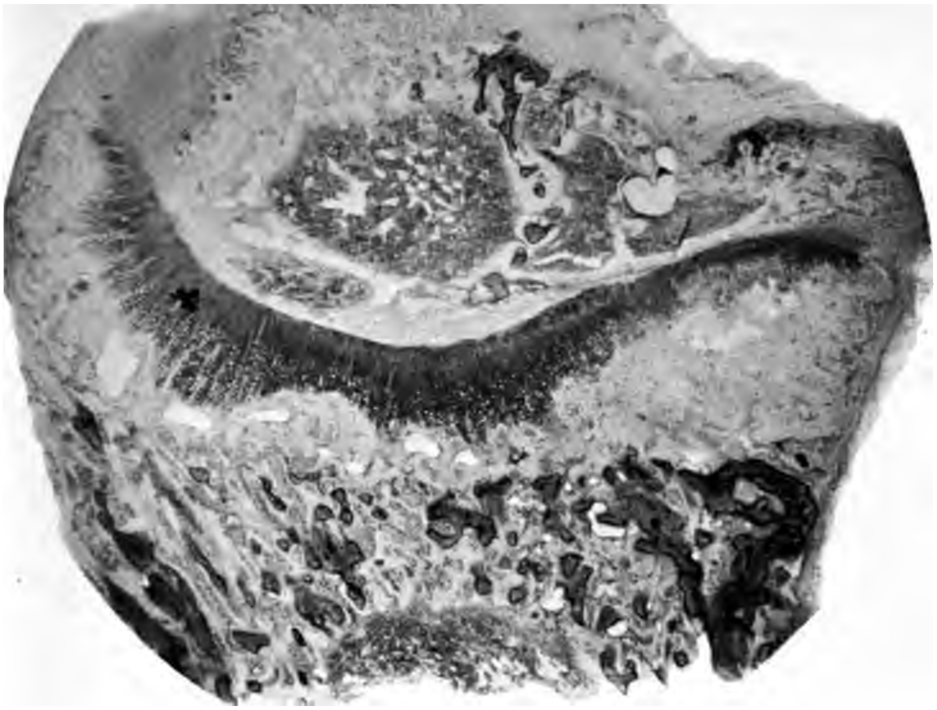

FIG. 3.

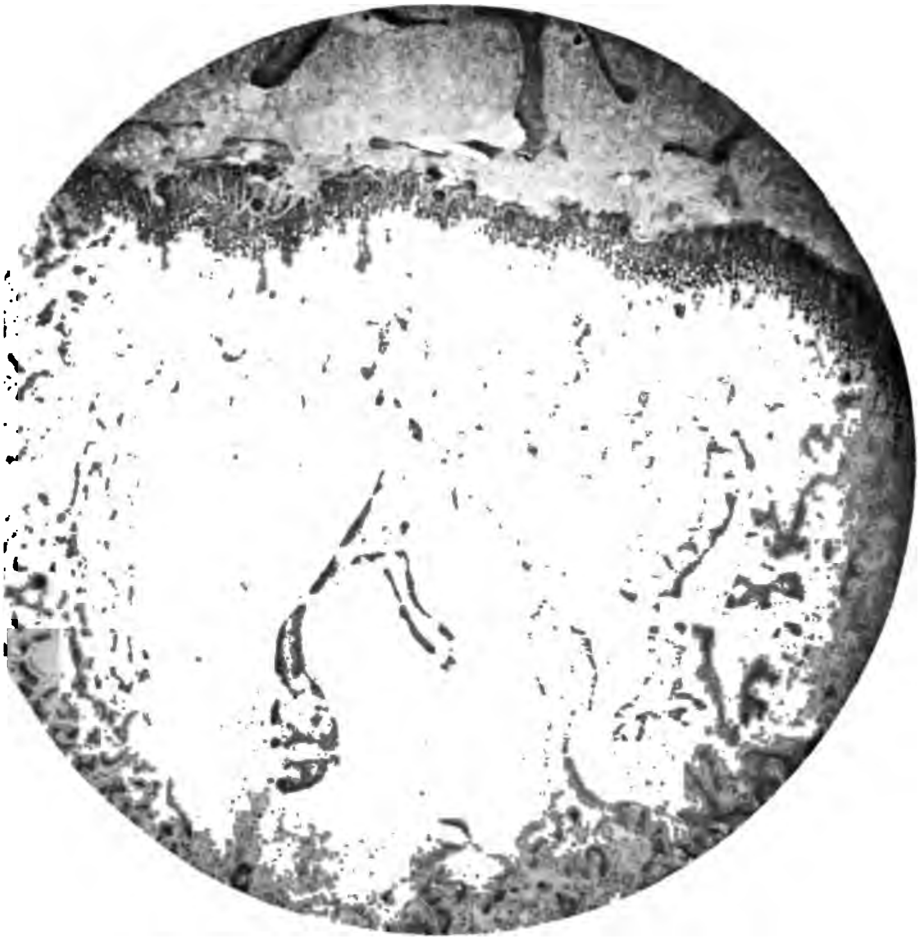

FIG. 4.

PLATE XXXII.

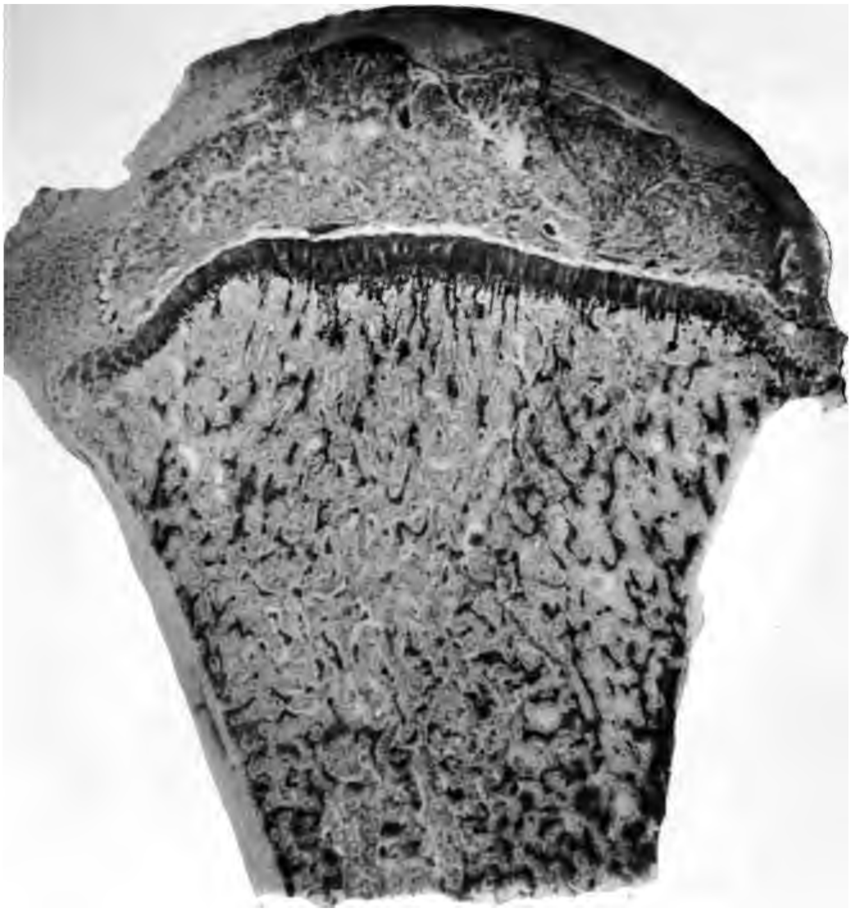

Fig. 5.

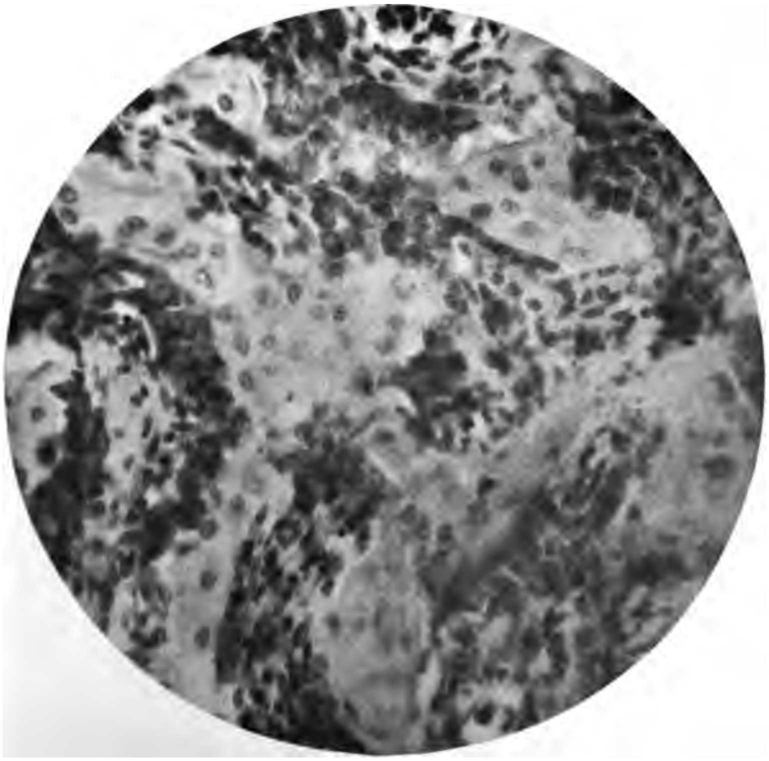

Fig. 6.

PLATE XXXIII.

growth, high fertility and the successful weaning of most of their young. The histological peculiarities of the bones of these rats are illustrated by the microphotograph in Fig. 3.

PLATE XXXI.

FIG. 2. This photograph illustrates the superior physical development and appearance of a group of rats fed the same diet as that of Lot 2821 (Fig. 1 and Chart 5), except that two per cent. of the butter fat in the latter was replaced by cod liver oil. The nature of the faults in the diet are described in the legend to Fig. 1 and that of Chart 5.

The bones of these animals were not far from normal in histological structure. The second generation had bones more different from the normal than were those of the first generation.

The appearance of these animals was strikingly superior to those restricted to the same diet containing 5 per cent. of butter fat. Cod liver oil contains some organic substance which has a pronounced anti-rhachitic effect, and protects young animals against the harmful effects of calcium deficiency and of unfavorable ratios between calcium and phosphorus.

PLATE XXXII.

FIG. 3. The distal end of the femur of a rat on a diet (No. 2810) low in calcium, but containing an amount of butter fat more than sufficient (5 per cent.) to protect the animal against keratomalacia. The cartilage is not calcified and the shaft of the bone contains large amounts of osteoid tissue.

FIG. 4. The bone shown in this figure is from an animal on the same diet (No. 2732) as that which furnished the bone in Figure 4, except that this animal received 1 per cent. cod liver oil instead of 5 per cent. of butter fat. The bone is somewhat osteoporotic, but both cartilage and trabeculae are heavily calcified and there is no osteoid present.

PLATE XXXIII.

FIG. 5. Lower end of the femur from a second generation animal on the diet of Lot 2733, to show the large number of very cellular trabeculae which are only partially calcified.

FIG. 6. Higher magnification of a portion of the bone shown in Figure 5. Note the extreme slenderness of the trabeculae and the large size and number of the osteoblasts, as well as the character of the osteoid tissue.

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STUDIES ON EXPERIMENTAL RICKETS.

VIII. THE PRODUCTION OF RICKETS BY DIETS LOW IN PHOSPHORUS AND FAT-SOLUBLE A.

By E. V. McCOLLUM and NINA SIMMONDS,

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

and P. G. SHIPLEY and E. A. PARK.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

Plates 4 to 7.

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INTRODUCTION.

In a brief preliminary report[1] we described a faulty ration and its effects on the skeleton of the growing rat, which corresponded in all essential details to rickets as that disease manifests itself in the skeleton of the human being. The faulty ration in question, No. 3127, was deficient in phosphorus and fat-soluble A.[2] In other respects it was, apparently, satisfactorily constituted. It contained protein of good quality and adequate in amount. The calcium was furnished in the form of the carbonate in a proportion (2 per cent of the total ration) which was essentially the amount necessary for the best promotion of growth, longevity, and reproduction. We took pains to point out that, when the content of phosphorus in the faulty ration was raised through the substitution of 2.5 per cent $CaHPO_4$ for the 2 per cent $CaCO_3$, osteoporosis and not rickets developed. We also called attention to the fact that, when a deficiency in calcium as well as in phosphorus was created through the omission of the $CaCO_3$ from the faulty ration, a pathological condition developed in the skeleton which was entirely distinct from rickets. We showed further that, when cod liver oil was added to the faulty ration in an amount equal to 2 per cent by weight of the ration, the pathological condition which developed in the skeleton was osteoporosis and had no resemblance whatsoever to rickets. We believe the results obtained in the experiments cited to be of considerable significance. In the first place, the experiments showed that it was possible to produce a pathological condition in the rat unquestionably similar to the rickets of the human being through the diet alone. In the second place, they showed that rickets could be induced by means of a ration, the faults of which were clearly defined and sharply limited; *viz.*, deficiencies in phosphorus and fat-soluble A. In the third place, they indicate that deficiency of phosphorus in the ration insufficiently supplied with fat-soluble A would give rise to rickets only when calcium was present in a ratio considerably higher than the calcium-phosphate ratio which is optimal for ossification. The experiments indicated, therefore, that the development of rickets in this instance depended on the existence in the faulty ration of (1) a specific disproportion in the calcium-phosphate ratio, the phosphorus being low, the calcium, relatively speaking, high, and (2) an insufficiency of an organic substance contained in cod liver oil having a profound influence on the calcification of cartilage and the ossification of bone.

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[1] Read before the American Pediatric Society, Swampscott, Mass., June 3, 1921.

[2] We are at present unwilling to commit ourselves to the view that the antixerophthalmic factor and the factor concerned in the ossification and growth of the skeleton are distinct, though we are prepared to acknowledge that such may be the case. We, therefore, use fat-soluble A to describe both the antixerophthlamic and antirachitic factors, assuming that the latter has a separate existence.

In the present paper we wish to describe further the composition of the faulty ration which produced the rachitic lesions above referred to, and to present in detail the anatomical evidence on which the statement of the production of rickets was based. We also wish to describe two other similarly constituted diets, one being the diet just referred to only slightly modified, and their effects on the growth and ossification of the skeleton in the rat.

Ration 3127.

	per cent
Rolled oats	40.0
Gelatin	10.0
Wheat gluten	7.0
NaCl	1.0
KCl	1.0
$CaCO_3$	2.0
Dextrin	39.0

This mixture was markedly deficient in phosphorus and in fat-soluble A (antixerophthalmic substance) but was otherwise well constituted. Its proteins were abundant and of good quality; its content of calcium not far from the optimum. So far as we can judge from experimental data available, the content of other inorganic constituents was satisfactory. Whileit did not furnish much above the actual requirement of the antineuritic substance, this factor could not be regarded as influencing in any way the well being of the animals.

TABLE I.

Data Concerning Rats of Lot 3127.

No. of rat.	Age when put on diet.	Weight when put on diet.	Days on diet.	Age at death.	Xerophthalmia.	Sex.	Weight.
	days	*gm.*		*days*			*gm.*
642	25	41*	24	49	+	♀	41
688	25	41	35	60	+	♂	48
689	25	41	35	60	+	♀	46
690	25	41	35	60	+	♂	46
708	25	41	39	64	+	♂	48

*The entire litter of rats composing Lot 3127 was weighed together. Each recorded weight in the column does not represent the exact weight of the individual but one-sixth the total weight of the litter. The sixth rat was partly eaten after being on the diet 35 days.

Ration 3133 was closely similar to Ration 3127 except that the former contained 0.5 per cent of butter fat in place of an equivalent amount of dextrin. This supplied a small amount of the fat-soluble A (antixerophthalmic substance) but not sufficient to entirely protect the animals against ophthalmia. This addition of butter fat was not sufficient to enable them to grow, although more liberal amounts would have done so. We know from other experiments, however, that they could not have been normal or have developed normal bones even with very liberal amounts of butter fat. The 0.5 per cent of butter fat added to their diet extended the lives of the animals to some extent.

TABLE II.

Data Concerning Rats of Lot 3133.

No. of rat.	Age when put on diet.	Weight when put on diet.	Days on diet.	Age at death.	Xerophthalmia.	Sex.	Weight.
	days	*gm.*		*days*			*gm.*
590	16	29*	35	51		♀	35
639	16	29	43	59		♀	27
706	16	29	59	75		♂	67
717	16	29	61	77		♀	45
811	16	29	91	107		♂	60
860	16	29	98	114		♂	39

*The entire litter of rats composing Lot 3133 was weighed together. Each recorded weight in the column does not represent the exact weight of the individual but one-eighth the total weight of the litter. Two rats disappeared from the cage; they were probably eaten.

Ration 3133.

	per cent
Rolled oats	40.0
Gelatin	10.0
Wheat gluten	7.0
NaCl	1.0
KCl	1.0
Dextrin	38.5
$CaCO_3$	2.0
Butter fat	0.5

Ration 3143.

	per cent
Wheat	33.0
Maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

This diet contains nearly twice the optimal content of calcium and is decidedly below the optimum in its content of phosphorus and of fat-soluble A. Otherwise it is well constituted. On this food mixture young rats grow somewhat more than on the other diets described in the paper. They do not develop xerophthalmia but become very deformed and die early.

When placed upon Ration 3127 the young rat lives for not more than 5 to 8 weeks. The growth momentum derived from the normal diet results in a preliminary gain. This soon ceases, however. The weight then remains stationary and declines toward the end of the experiment. The rats of Lot 3127 were 25 days old when confined to the faulty ration, and were killed after periods varying from 24 to 39 days.

TABLE III.

Data Concerning Rats of Lot 3143.

No. of rat.	Age when put on diet.	Weight when put on diet.	Days on diet.	Age at death.	Xerophthalmia.	Sex.	Weight.
	days	*gm.*		*days*			*gm.*
571	50	64	50	100		♂	81
657	50	65	63	113		♀	82
658	50	67	63	113		♀	82
831	45	76	43	88		♂	62
879	40	60	60	100		♂	65
914	40	55	65	105		♂	68
915	40	53	65	105		♂	75
918	40	60	76	116		♀	60
919	28	56	69	97		♀	63

At autopsy the bodies were small and greatly emaciated. Almost no fat was present. The eyes were inflamed. The incisor teeth were brittle. The thorax was flattened at the sides, and in one or two animals was hollowed slightly along the line of junction of the cartilages and the ribs. In none of the animals, however, was it greatly deformed. The costochondral junctions themselves formed fusiform enlargements, most evident on the internal surface of the thorax. The enlargements were thrown into especial prominence if the thoracic wall was bent inwards. Fractures of the shaft of the ribs with callous formation situated usually not far behind the costochondral junctions were present in three of the animals. The lower end of the radius and ulna and the adjacent ends of the femur and tibia formed enlargements similar to those seen in the rachitis of human beings. When stripped of their muscles the ends of all the long bones were found to be enlarged. Fractures of the long bones of the extremities were not noted. The strength of the tibia and femur was greatly reduced and both these bones and the ribs offered little resistance when cut. Examination of the cut surface of the femur or tibia showed that the junction of cartilage and shaft was not clearly defined and that below the cartilage there lay a pale yellow zone of not very great depth which proved when examined under the microscope to be a transitional zone containing a mixture of elements derived from cartilage and shaft. Examination of the cut surface further showed the cortex to be extremely thin and the bone marrow to have approximately normal color.

The following changes were noted when the bones were examined under the microscope.[3] The epiphyseal cartilage separating the nucleus of ossification from the shaft was in most places broader than in the healthy animal and at several points was continued in irregular blue-staining prolongations toward or actually to the shaft. Its diaphyseal border did not end abruptly on coming in contact with the elements derived from the diaphysis but seemed to undergo a gradual transition into the latter. The zone of undifferentiated cartilage was represented by few cells. Almost at their beginning, close to the nucleus of ossification, the cells of the epiphyseal cartilage became arranged in columns or fascicles separated by rather more matrix than is seen in the bones of young rats on normal diets. Near their points of origin the cells composing the columns were flattened as if compressed in the long direction of the shaft and lay close to each other, but at a short distance from their points of origin became oval, round, or cuboidal. As soon as the cells of the epiphyseal cartilage underwent the expansive change just referred to, the intercellular substance which separated the cell groups became greatly diminished, so that the columns appeared to lie close to each other. Coincidently, the cells seemed to lose to a certain degree their affinity for hematoxylin with the result that both nucleus and cell body appeared more pale. The cell membrane appeared to be unusually thick. In general the nuclei were large, round, and well preserved. The columnar arrangement of cells was at some points maintained throughout the breadth of the cartilage, but at others became irregular or entirely lost as the metaphysis was approached, so that the cells seemed to be irregularly jumbled together. Wherever the cartilage cells came in proximity to the marrow elements they lost their blue color and were pale yellow. In many places the cartilage after losing its staining reaction ended abruptly in contact with the blood vessels derived from the marrow. At other places, however, the cartilage was continuous with the cartilage which formed the bulk of the transitional zone between cartilage and shaft, the rachitic metaphysis (Fig. 3).

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[3] The technique employed in these experiments was as follows: The bones were hardened in formaldehyde. They were cut after embedment in celloidin in sections 15 to 25 mikra thick and stained with hematoxylin and eosin. Control preparations were made with the freezing microtome and stained with hematoxylin and also silver nitrate after the method of von Kossa.

The metaphysis in which the elements derived from cartilage and shaft were blended in an irregular manner was not deep. Indeed in comparison with the metaphysis in the bones of the rats on Rations 3133 and 3143, it was exceedingly shallow. It was composed of the irregular prolongations of cartilage from the main mass which retained their blue stain, and of quantities of cartilage cells which had lost their staining reaction to hematoxylin entirely and were in all stages of transition into osteoid. There were osteoid trabeculæ derived largely from cartilage and having a waxy appearance. The remains of calcified intercellular substance staining deep blue and having a configuration like the cross-section of a honeycomb were formed in the metaphysis. The detailed description of the various elements composing the metaphysis will be found in the description of the histological findings of the rat on Ration 3133 (Fig. 4).

The cortex of the shaft near the metaphysis was broad and composed of a thick interlacing network of trabeculæ having for the most part central cores of calcified material and borders of osteoid. As the middle of the bones was approached, however, the cortex became thin. The trabeculæ had a central core of calcified material but peripheral portions composed of osteoid. The osteoid zones were fairly broad. In most of them a fibrillary arrangement could be made out. The bone corpuscles of the calcified portion were small and those in the osteoid borders appeared especially small and far separated from each other. They presented a variety of shapes. Some were round, some spindle-shaped, some irregular. Moreover, they were very unevenly distributed. Some resorptive phenomena could be recognized in the calcified portions of the trabeculæ. The marrow appeared normal. The nucleus of ossification showed changes both in its cartilaginous elements and in the trabeculæ exactly analogous to those described in the epiphyseal cartilage, and in the shaft.

The rats on Ration 3133 were younger (only 16 days old) when placed upon the faulty mixture than the rats of the lot on Ration 3127, and were kept on the faulty diet for longer periods of time, on the average 63 days. The shortest period was 35 days and the longest 98 days. The rats scarcely more than maintained their initial weight on Ration 3133. They were small and poorly nourished. The eyes of most of them were inflamed. The thorax was considerably deformed. The costochondral junction was considerably enlarged. In the majority of the animals they were displaced inwards and were greatly distorted. Fractures of the ribs were present with well marked callous formation. The knees, wrists, and ankles were considerably enlarged, as were the ends of all the long bones. The femurs and tibias cut with greatly diminished resistance. On section the enlargement of the ends of the long bones was especially evident and seemed to correspond to the cartilage and to a white zone between the cartilage and bone marrow which on microscopic examination proved to be the rachitic transitional zone or metaphysis. The line of junction between the cartilage and shaft was indistinct. The shafts of the long bones were not enlarged. The spleen was not increased in size in the majority of the rats but was enlarged in some.

The microscopic changes in the femurs and the tibias of the rats on this diet resembled those described in the bones of the rats on Ration 3127, but were considerably further advanced. The epiphyseal cartilage was irregularly increased in depth and seemed to merge with the metaphysis in a manner presently to be described. The breadth of the metaphysis was almost if not quite equal to the breadth of the epiphyseal cartilage. The shaft of the bone was broad where it bordered on the metaphysis but rapidly contracted so that the shaft of the bone as a whole did not seem to be enlarged (Fig. 2).

The undifferentiated cartilage was represented by only a few scattered cells or cell clusters. Almost at their beginning the cartilage cells became arranged in short columns or rather groups of columns. These were more widely separated from each other by matrix than in the healthy animal; that is, the amount of matrix seemed to be considerably in excess of that found in the rat under normal conditions. The columns or bundles of cartilage cells just referred to took their origin at somewhat different levels. They were bullet-shaped and the ends of the columns directed toward the nucleus of ossification were for the most part pointed. The cartilage cells composing them were flat, the long dimension running from side to side. Some of them had the same thickness throughout, others were spindle- or wedge-shaped. Many of them were curved like crescents, the concave side of the crescent opening diaphysealwards. The nuclei were in some instances centrally placed, in others at the side. The nuclei were long and thin, partaking of the shape of the cell, and took a very dark stain with hematoxylin. In some cells no cytoplasm could be seen; in all, the cytoplasm if present was scant and took a paler blue stain than the cell capsule or nucleus. The cell capsule was not clearly defined and could not be easily separated from the adjacent cell capsules or surrounding matrix. In most of the bundles the arrangement of the cartilage cells was quite irregular. The cells lay one upon the other, but not in continuous columns. One row of cartilage cells would appear to interdigitate with another or a third column would seem to be interpolated between two rows of cartilage cells. The matrix in the part of this portion of the proliferative cartilage near the nucleus of ossification stained blue with hematoxylin but as the hypertrophic zone of the cartilage was approached it began to lose its blue color and appeared yellow.

As the metaphysis was approached the cells of the cartilage underwent a remarkable change. The cells forming the bundles suddenly expanded assuming globular, oval, or cuboidal shapes and at the same time began to lose their power to take the blue stain of the hematoxylin and soon ceased to take it altogether. The nuclei shared in the expansion of the cells, becoming large, round, and vesicular, and took a pale blue stain. The matrix between the cell bundles which had lost its blue color earlier than the cells themselves was stained a pale yellow after hematoxylin and eosin. As the cells increased in size, the broad zones of matrix which separated them became somewhat decreased in amount with the result that the columns lay closer to each other. In many places, however, the broad zone of matrix which separated the bundles of flat cells above continued to exist between the columns of swollen cells nearer the diaphysis. As the cartilage cells underwent the sudden enlargement and alteration in staining reaction their columnar arrangement tended to become more definite. In certain places in which the cartilage was prolonged in solid masses into the metaphysis, the cells tended to retain their affinity for hematoxylin. The morphology of the cells in these irregular prolongations will be discussed later. *Calcification of the cartilage was entirely wanting in all the animals on this diet.*

The metaphysis varied considerably in its depth, and in its general character. In the majority of the animals it was deep. In one rat, however, which weighed only 25 gm. when killed, it was shallow. In all the animals it was made up of the various elements derived from cartilage and shaft which usually compose the metaphysis of a rachitic bone intermingled in an irregular manner. There were the irregular prolongations of the epiphyseal cartilage just referred to which retained their staining reactions to hematoxylin. There was cartilage which had lost its staining reaction altogether and seemed to be in various stages of transformation into osteoid. Many blood vessels were present, filled with red blood cells and surrounded by marrow elements. There were remnants of calcified matrix and in some rats considerable amounts of fibrous tissue (Fig. 5).

In all of the rats by far the greater part of the metaphysis was composed of the cartilage described as having lost its staining reactions entirely in the process of transformation into osteoid. This cartilage appeared in the sections lightly colored as cream color or pale yellow with eosin. It had exactly the same staining reaction as the osteoid borders of the trabeculæ. These cartilage cells for the most part looked swollen and presented a great variation in size, shape, and general morphology and composed the main bulk of the metaphysis. Many were large, and round, oval, or cuboidal in shape. Some were pear-shaped and some flattened. The nuclei were for the most part large and round and stained pale blue but in some the nuclei were small and deep blue or pycnotic. The cell bodies were yellow like the surrounding matrix or were actually colorless. The capsules appeared to be exceedingly thin. They were colorless in the case of many of the cells and could be distinguished only with reduced light as a transparent circle surrounding the cell. In the case of other cells the capsules retained some of the blue stain. The cartilage cells which had thus lost their staining reaction were anything but uniform. A row of globular cells like those just described might be continuous with a group of small cells, the smallest perhaps no larger than osteoblasts with correspondingly small nuclei staining deeply with hematoxylin, or they might be continuous with cells having a flat contour like those described in the cell bundles near the epiphyseal nucleus. Cells could be found which seemed to be fading away into a substance indistinguishable from the surrounding matrix. No nuclei seemed to be present in them, or the nuclei were so faintly stained as to be scarcely visible and the existence of the cells could be recognized only by the faint outline of the cell body visible under reduced illumination. In general, the cells in the metaphysis near the cartilage tended to be disposed in columns which were continuous with the columns of the cartilage proper. On the diaphyseal side of the metaphysis, however, the columnar arrangement had very largely disappeared. The large globular forms of cartilage cell were more numerous near the epiphyseal cartilage. The small forms were especially common on the diaphyseal side. The degenerating forms, also much more frequent on the diaphyseal side, were especially numerous in the immediate neighborhood of the blood vessels. Some of the blood vessels seemed to be surrounded by matrix exactly like the osteoid covering the trabeculæ in its staining properties and almost devoid of nuclei. The amount of matrix in which the cartilage cells lay was present in much greater quantity on the diaphyseal side of the metaphysis than on the side of the epiphyseal cartilage (Fig. 7).

The cells of the cartilage which retained their staining property were for the most part those composing solid prolongations from the main mass of epiphyseal cartilage. Blood vessels did not penetrate them. The retention of their normal staining properties and a more nearly normal morphology may have been due to the fact that they happened to have been protected from the influences of the diaphyseal circulation. The cells composing these irregular prolongations, however, showed considerable morphological variation. Near the epiphyseal cartilage they were frequently indistinguishable from the large globular cells close to the bundles of flattened cells. As the diaphysis was approached, however, the cells perhaps assumed a flat shape and appearance exactly comparable to the cells forming the bundles at the beginning of the epiphyseal cartilage. Nearer the diaphysis they might again become large and round and finally they might again become flat or show evidence of partial calcification or break up into small groups composed of cells globular in form and much reduced in size, with clearly defined rather thick blue staining cell capsules and separated by an abundant matrix which had lost its staining reactions to hematoxylin altogether. Blood vessels penetrated the metaphysis from the shaft, and were found at the level at which the sudden transition in the cartilage above described took place; *i.e.*, to the level at which the cartilage cells suddenly became swollen and lost their staining properties. This fact would seem to indicate that the changes in the cartilage might be initiated as the result of circulatory influences. Blood vessels of all sizes could be seen ramifying in an irregular manner through the diaphysis. For the most part, however, they were not large. The large vessels terminating in huge tufts so frequently seen in the bones of rachitic human beings were not present. The blood vessels were filled with red blood cells and were surrounded by marrow elements. In general, it would seem that they penetrated the cartilage rather by insinuating themselves along the intercellular ground substance separating the columns than by destruction of the cartilage cells themselves. Occasionally, however, places could be found in which the red blood cells were present within the cartilage cell capsule. Evidently under the abnormal conditions induced by the diet the cartilage cells could be destroyed by the vascular elements, but in general tended to persist, undergoing a slow degeneration or change into hyaline substance or possibly a change of a metaplastic nature into osteoid or connective tissue. In general, the blood vessels had a delicate endothelial lining but groups of red blood cells could be found lying in spaces in the metaphysis without evidence of surrounding endothelium. In general, the metaphysis did not appear to be disrupted by the vascular elements to the extent frequently seen in the bones of rachitic human beings.

Though in none of the bones of the animals on this diet was there calcium deposition in the cartilage, in almost all there were irregular calcium deposits at one point or another in the metaphysis. In one animal a zone of calcium deposition extended completely across the metaphysis, bisecting it. The calcium deposits were delicate and for the most part completely surrounded the cartilage cells, having in sections an appearance like that seen in a cross-section of a honeycomb. The cartilage cells in the metaphysis encased in calcified matrix showed morphological changes and loss of staining reactions comparable to those in evidence in the cartilage of the metaphysis elsewhere. In some of the interstices of the calcified matrix red blood cells and other marrow elements could be seen.

In one or two of the rats there was considerable connective tissue formation exactly similar to the fibrous tissue commonly seen in the rickets of human beings. As the shaft was approached the numbers of the blood vessels increased and the numbers of cells of diaphyseal origin became more numerous. Some of them bordering the blood vessels had the morphology of osteoblasts, others of connective tissue cells. Trabeculæ devoid of any calcified matter but containing instead cores of cartilage cells which varied greatly in size and were in various stages of transformation or degeneration into osteoid were seen in the shaftward portion of the metaphysis. The junction of the diaphysis and metaphysis in most of the rats was marked by calcified intercellular substance indicating the level in the growing end of bone at which the effect of the faulty diet first became manifest.

The cortex was thin and the trabeculæ were covered with osteoid. The cells of the osteoid investments were for the most part elongated, resembling connective tissue cells, and were widely separated from each other. The osteoid had a fibrillar arrangement. In some of the animals there were large osteoid formations on one side of the bone or the other under the periosteum. Few evidences of resorptive activity could be found. The marrow elements appeared to be essentially normal (Fig. 6).

In experiments with the rats of Lot 3143 the normal diet was stopped and the faulty ration substituted at ages ranging from 28 to 50 days. The animals were allowed to live on the faulty ration for from 43 to 76 days. When placed upon the faulty ration they continued to gain slowly or remained stationary in weight. They never developed inflammation of the eyes (xerophthalmia) on account of the presence of sufficient protective substance in the wheat and maize of the diet. If this diet is continued over a sufficient length of time the animals die. Just before the end of their lives rats on this ration show a definite loss of control of the posterior extremities which results in a tottering gait. The trouble is progressive and finally results in a complete paralysis of the hind legs. This condition will be fully discussed in another communication.

At autopsy they were small and emaciated. The incisor teeth were loose and so brittle as to be easily fractured. The thorax was greatly deformed. Its anteroposterior diameter was greatly increased as compared with its lateral diameter and the lower part of the sternum projected forward as in the pigeon breast deformity of human beings. At the line of the costochondral junctions there were deep grooves. When the thorax was opened and the interior examined the deformity was enormous. The costochondral junctions and shafts of the ribs met at acute angles, the apices of which pointed toward the vertebral column and were not far from it. The costochondral junctions themselves were exceedingly large and some of those of the lower ribs were twisted into the most bizarre shapes. The posterior epiphyseo-diaphyseal junctions were enlarged and appeared like so many white beads on either side of the spinal column. Many fractures of the shafts of the ribs were present, marked by large white callous formations. The spinal column itself was bent in various curvatures. The wrists, knees, and ankles were greatly enlarged, as indeed were the ends of all the long bones. Even the scapulæ were deformed. Fractures of the tibia marked by angular deformity and huge callous formation were present in some of the rats at the junction of the upper third with the lower two-thirds.

The description of the microscopic changes in the bones of the animals on this diet corresponds in its main features to the description already given of the microscopic changes in the rats on

Ration 3133. We shall limit the description of the microscopic changes, therefore, to a few observations. The enlargement of the ends of the long bones was due to an increased depth and probably also to an increased breadth in the epiphyseal cartilage and to the presence of an enormous metaphysis. The width of the metaphysis was equal to that of the epiphyseal cartilage; its depth was many times greater. The groups of flattened cartilage cells constituting the columnar zone of the cartilage were very widely separated from each other by hyaline matrix. It was impossible not to think that the amount of hyaline matrix throughout the cartilage was greatly increased. The hyaline matrix early lost its power to take the hematoxylin stain. As soon as the cartilage cells had undergone the expansive change fully described in the case of the rats on Ration 3133 they rapidly lost their normal staining properties and underwent the changes of a metaplastic and degenerative character already described. By far the greater part of the metaphysis was composed of cartilage or derivatives from the cartilage. The osteoid trabeculæ of the metaphysis in the main had a cartilaginous basis; that is, in almost all of them cartilage cells in various stages of degeneration or metaplasia could be identified. The cartilage in every animal of the group was entirely free from lime salt deposits. Some irregular lime salt deposition was present in the metaphysis of certain of the animals. The blood vessels which ramified through the metaphysis were in the main small. The cortex was thin. It was composed of bone-containing scanty central cores of calcified material and with broad osteoid borders. The breadth of the osteoid borders was extreme. The bone corpuscles found in them were widely separated from each other by quantities of yellow osteoid material. The number of cells per unit of area was consequently greatly diminished. The nuclei were long, some were spindle-shaped. They resembled the nuclei of connective tissue cells much more closely than those of bone corpuscles. A fibrillar arrangement of the osteoid could be made out. Few osteoblasts or at least cells which could be identified as such could be found lining the osteoid trabeculæ. The evidences of resorption of the calcified portions of the trabeculæ were slight. The bone marrow appeared to be essentially normal (Fig. 1).

Osteoid production was extreme in the epiphyseal nuclei of ossification. The large calluses which were formed about healing fractures were composed entirely of osteoid tissue.

DISCUSSION.

The faulty rations, Nos. 3127 and 3133, containing 2 per cent added calcium carbonate, gave rise to pathological conditions in the skeleton essentially identical with those found in the human subjects of rickets. The rachitic lesions in the group of rats on Ration 3133 were more advanced than those in the group of animals on Ration 3127, presumably on account of the presence in Ration 3133 of 0.5 per cent of butter fat. This small quantity of butter fat, while insufficient to prevent the development of xerophthalmia, was sufficient to delay its advent and to diminish its rate of progress. It increased the duration of life and made possible, presumably, increased growth of the skeleton. The small amount of butter fat in Ration 3133 probably intensified the rickets-producing properties of the diet through its stimulating effect on growth and its favorable influence on the duration of life. We have already called attention to the fact that butter fat as compared with cod liver oil is exceedingly low in its rickets-inhibiting properties.[4]

Ration 3143 was derived in part from wheat and maize, which contained a certain amount of antixerophthalmic substance (more than was present in Ration 3133, which contained 0.5 per cent butter fat). Ration 3143 further differed from Rations 3127 and 3133 in having a higher calcium-phosphate ratio than either. It gave rise to the most extreme degree of rickets. The osteoid production and the metaplastic and degenerative changes in the cartilage exceeded anything ever seen in the bones of rachitic human beings. We have repeatedly observed that, if calcium carbonate in large quantities (3 to 6 per cent of the total ration) is added to a ration insufficiently supplied with the organic factor and only slightly or not at all deficient in its content of phosphorus, most pronounced changes occur at the growing ends of the long bones. The cartilage undergoes the degenerative and metaplastic changes already described, and fails to take up calcium phosphate with any regularity, or to take it up at all. The trabeculæ near the end of the shaft become surrounded with enormous quantities of osteoid and great quantities of osteoid trabeculæ largely derived from the cartilage develop between the cartilage and the shaft. The exaggerated character of the rachitic lesion brought about by Ration 3143 is explicable on the ground that the phosphate is extremely low and the calcium high (3 per cent of the total ration in the form of calcium carbonate), so that the calcium-phosphate ratio was exceedingly high. The quantity of the antixerophthalmic substance contained in Ration 3143 was sufficient to prevent effectually the development of xerophthalmia. The quantity of the antixerophthalmic substance contained in the diet, however, was insufficient to exert, or incapable of exerting, any visible inhibitory influence on the development of the rachitic lesions in the skeleton. Indeed, it seems not unlikely that the amount of the organic factor contained in the wheat and maize of Ration 3143 may have intensified the development of the rachitic changes by promoting the growth of the skeleton and increasing the duration of life of the animal, as did the small amount of butter fat in Ration 3133.

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[4] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. *Proc. Soc. Exp. Biol. and Med.*, 1920–21, xviii.

In a preliminary communication[5] Sherman and Pappenheimer described the production of rickets in rats by means of diets containing patent flour, 95 per cent; calcium lactate, 3 per cent; and sodium chloride, 2 per cent, with and without the addition of 0.1 per cent ferric citrate. They also described the prevention of the rickets when potassium phosphate in amounts equaling 0.4 per cent of the total was added. They also observed that, when no calcium was added to their faulty rations, osteoporosis instead of rickets developed. Patent flour is one of the most deficient foods which enters into the human diet, being exceeded in this respect only by isolated foods such as starch, sugars, fats, or polished rice. Bolted flour is rather poor in protein and this is of rather poor quality. It is very deficient in calcium, phosphorus, sodium, chlorine, iron, and possibly also in potassium. The only essential inorganic element which it probably contains in amount sufficient to meet the physiological needs of an animal is magnesium. Bolted flour is also very deficient in the antineuritic substance (water-soluble B) as shown by experiments on animals and by the frequent occurrence of beri-beri in man in Labrador and Newfoundland, where bread from this source is a principle article of food. It is exceedingly poor in fat-soluble A, and in the organic antirachitic factor. The lack of antiscorbutic substance in flour is, we believe, a matter of little or no importance in the nutrition of the rat, since this species is capable of synthesizing this complex. The ration employed by Sherman and Pappenheimer was, therefore, deficient in fat-soluble A, water-soluble B, its protein content, potassium, phosphate, and water-soluble C. In the presence of so considerable a number of defects in the ration it was impossible to be sure which were operative in the production of the disease. In the light of our experience it seems probable that their results should be interpreted as follows: The development of the rachitic condition in their animals was due (1) to a disproportion in the calcium-phosphate ratio, the calcium being present in optimal proportion or in a proportion not far from the optimal (3 per cent of the *lactate* of calcium) and the phosphorus at the lowest possible level, and (2) to a deficiency of the preventive organic substance. When neither calcium nor phosphate was added, the ratio between these (in the patent wheat flour) was more nearly the optimum than after the calcium addition, and, as was to be expected, osteoporosis, not rickets, developed. The animals needed (among other things) calcium very badly; yet we witness here the apparent paradox that meeting a physiological need and without exceeding it (*i.e.* by adding to the diet 3 per cent calcium lactate) more damage of a particular kind is done than would have resulted from permitting the pronounced calcium starvation in addition to the other deficiences of their experimental diet to continue. *Apparently in the rat the profound disturbances in the deposition of lime salts in cartilage and bone and the changes in the cells of those tissues which give rise to the pathological complex known as rickets may be produced by disturbances in the diet of the optimal ratio between calcium and phosphorus in the absence of an amount of an organic substance contained in cod liver oil sufficient to prevent them. It would seem from the results of a large number of experiments, which will be published in detail soon, that in so far as calcium and phosphate are con-*

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[5] Sherman, H. C., and Pappenheimer, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1920–21, xviii, 193.

cerned, the physiological relation in the diet between the two is of infinitely greater importance in insuring normal calcification than the absolute amount of the salts themselves.

In examining the microscopic preparations from the rats which served as the material for the experiments reported in this paper, we were again struck with the similarity of behavior of the cartilage cell and of the bone corpuscle under the abnormal conditions imposed by the faulty diets. On coming into direct contact with the blood vessels the cartilage cell tended to undergo a gradual degeneration or transformation into a homogeneous substance indistinguishable except in its lack of a fibrillar arrangement from the osteoid zone surrounding the calcified trabeculæ. On coming into less direct contact with the vascular elements the cartilage cell tended to revert to its original state in the undifferentiated cartilage, or to some intermediary state of development, or to undergo metaplasia into bone corpuscles, at least such as are found in the osteoid, or into connective tissue. In the process of reversion or of metaplasia of the cartilage cell in which it underwent a diminution in size, lost its characteristic staining reaction to hematoxylin, etc., it surrounded itself with (manufactured), or was surrounded by, an increased amount of homogeneous matrix, which remained free from lime salt deposition. The bone corpuscles in contact with the vascular elements, *i.e.* those of the osteoid borders of the trabeculæ, underwent a diminution in size and a change in shape, and tended to revert, if they actually did not do so, to connective tissue cells. At the same time they surrounded themselves with (manufactured), or were surrounded by, an excessive amount of matrix, which remained free from lime salt deposition. In our experience whatever the reaction of the osteoblasts to the abnormal condition of the faulty diet, the same reaction will be manifested by the cartilage cell. The processes of bone resorption so prominent in the skeleton of rats confined for periods of some duration to faulty diets low in calcium seemed to be held in abeyance under the particular abnormal conditions obtaining in these experiments. Only slight evidences of resorptive activity in the calcified portions of the trabeculæ could be found. In the presence of the particular disproportion in the calcium-phosphate ratio characterizing the faulty diets used in these experiments both osteoblast and cartilage cell seemed to undergo changes which made them particularly resistant to the destructive forces ordinarily in operation.

EXPLANATION OF PLATES.

PLATE 4.

FIGS. 1 and 2. Photomicrographs of low magnification of sections from the distal end of the femur of rats on the diet of Lots 3143 and 3133. The photomicrographs show the appearance of bones affected with a very severe rickets. There are broad zones of osteoid tissue around the trabeculæ and the epiphyseal nucleus of ossification and the diaphysis. The cartilage is persistent to a marked degree, forming a wide metaphysis. It contains no calcium salts and is invaded by blood vessels from the shaft. (Rats 679 and 811. Diet of Lots 3143 and 3133.) Objective—Leitz microsummar F-5 full aperture. No ocular.

PLATE 5.

FIG. 3. This picture shows a condition which exactly simulates that found in mild cases of rickets in man. The osteoid borders of the trabeculæ in this bone are much narrower than those of the bones shown in Figs. 1 and 2. The cartilage is persistent and irregularly invaded. There is no zone of provisional calcification. The metaphysis of the bone is narrow and contains small scattered deposits of calcium salts. This picture was taken with the same apparatus used to photograph Figs. 1 and 2. (Rat 642. Diet of Lot 3127.)

FIG. 4. To show detail of the growing region of the bone in Fig. 3. Note the absence of provisional calcification and the irregular prolongation and invasion of the cartilage. The metaphysis, which is narrow, is made up of trabeculæ of osteoid, and the metaphysis contains irregular deposits of lime salts. Objective—Leitz acromatic No. 3. No ocular.

PLATE 6.

FIG. 5. Photomicrograph with the same apparatus used in making Fig. 4. This picture shows the metaphysis in detail of a bone affected with exaggerated rickets. The very broad metaphysis is composed of osteoid trabeculæ, traversed by a few small marrow spaces, and blood vessels which have sprouted from the vascular tree of the diaphysis. Much of this osteoid tissue was the product of metaplasia of the cells of the epiphyseal cartilage, some of which may be seen embedded in it in a condition of transition into osteoid corpuscles. (Diet of Lot 3133.)

FIG. 6. High power photomicrograph. This picture shows calcified bone, *OS*, surrounded by a zone of osteoid tissue, *OST*. Note the lamination of the osteoid, the small size and wide separation of the osteoid corpuscles and the endothelioid appearance of the osteoblasts which surround the trabeculæ, *O*. (Diet of Lot 3143.) Objective—Leitz acromatic No. 6. No ocular.

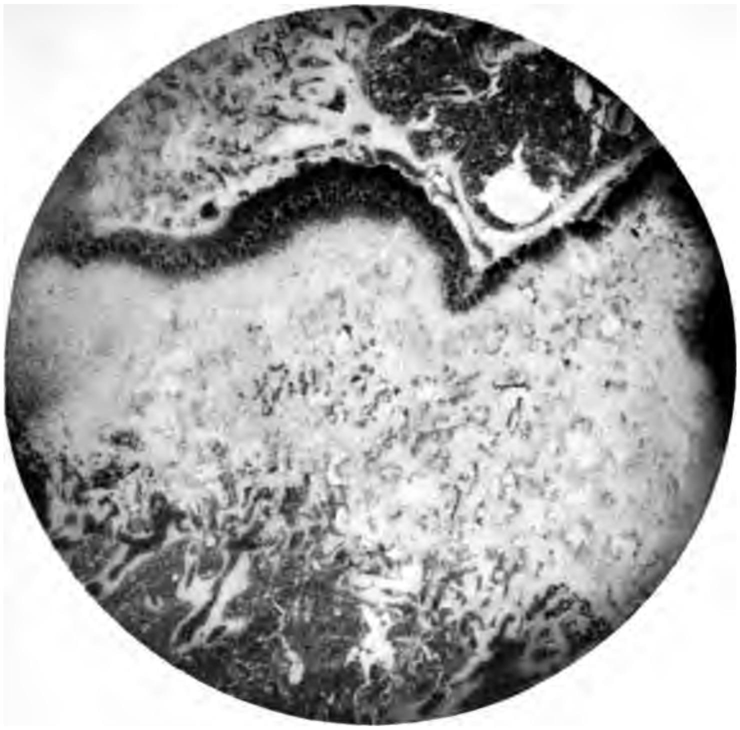


FIG. 1.

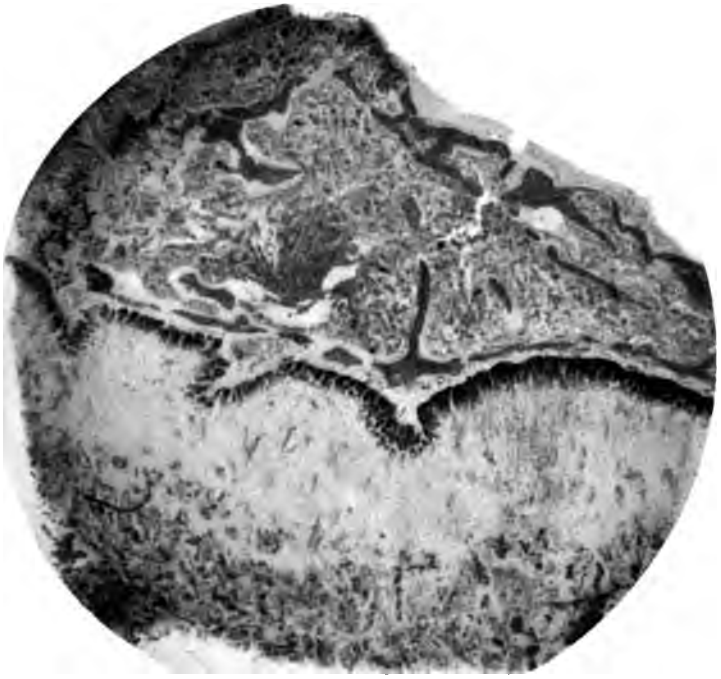
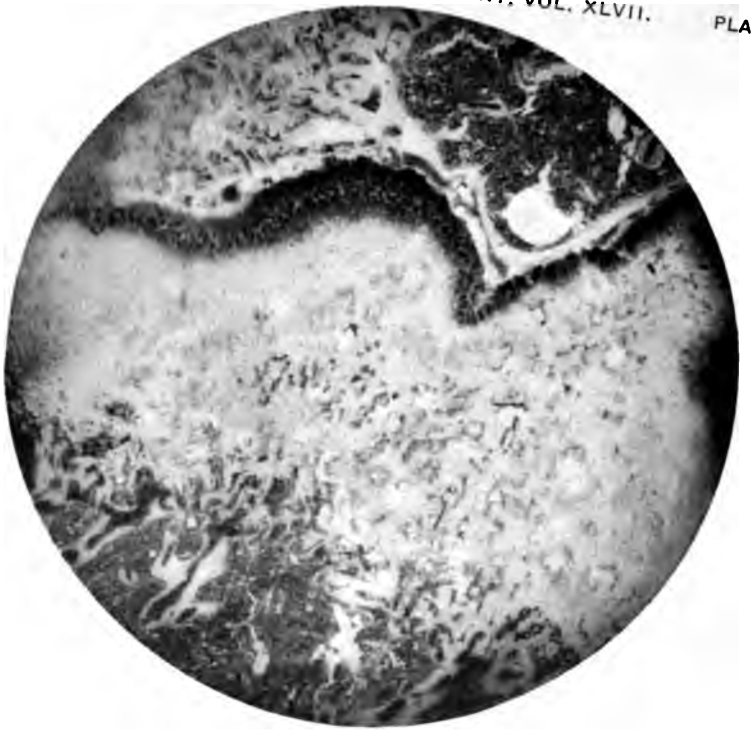


FIG. 2.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. VIII.)



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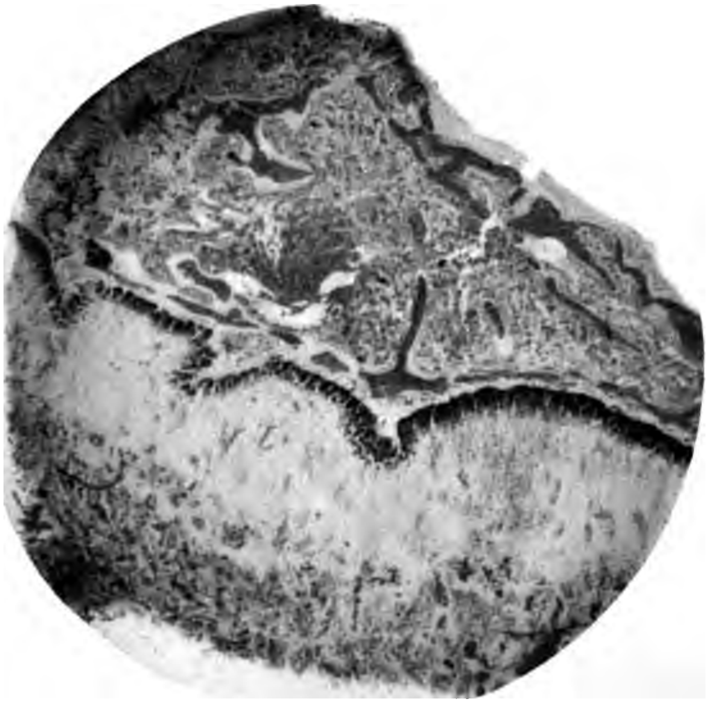

Fig. 2.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. VIII.)

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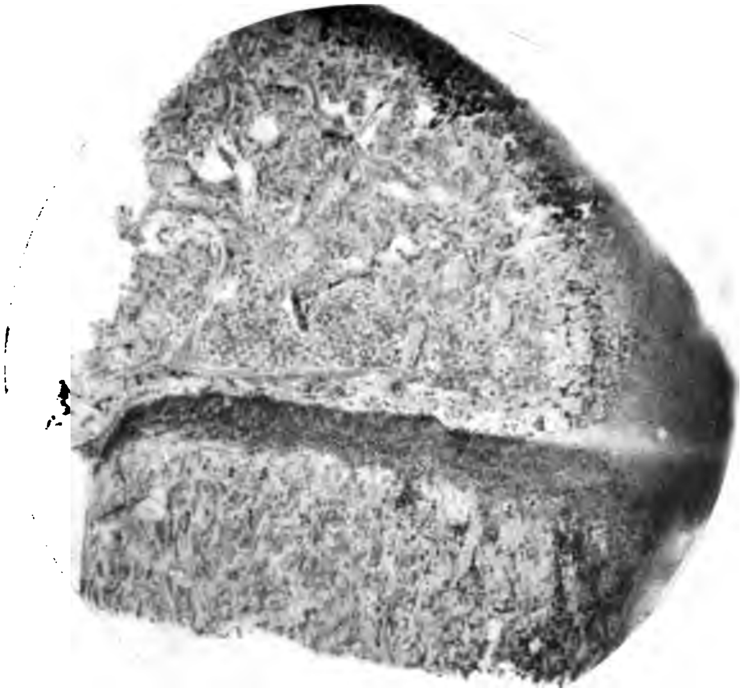

FIG. 3.

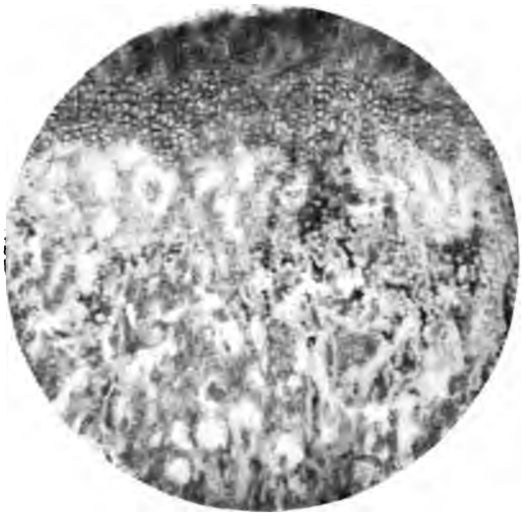

FIG. 4.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. VIII.)

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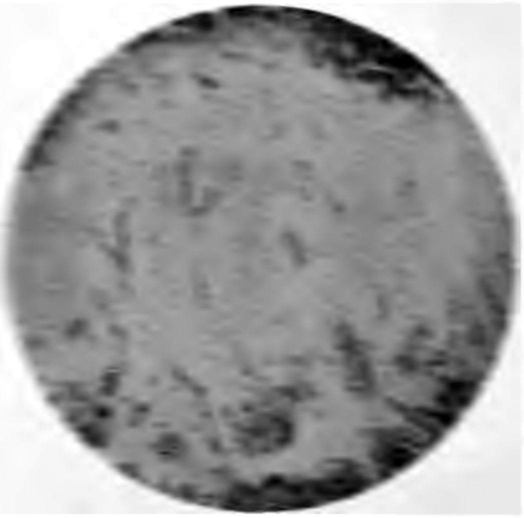

Fig. 5.

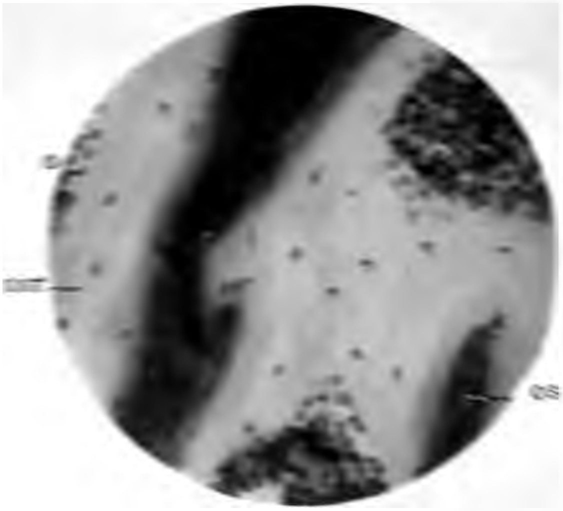

Fig. 6.

McCollum, Simmonds, Shipley, and Park: Experimental rickets. VIII.

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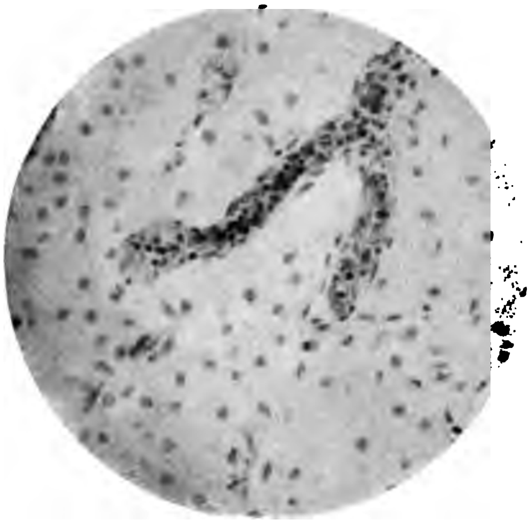

FIG. 7.

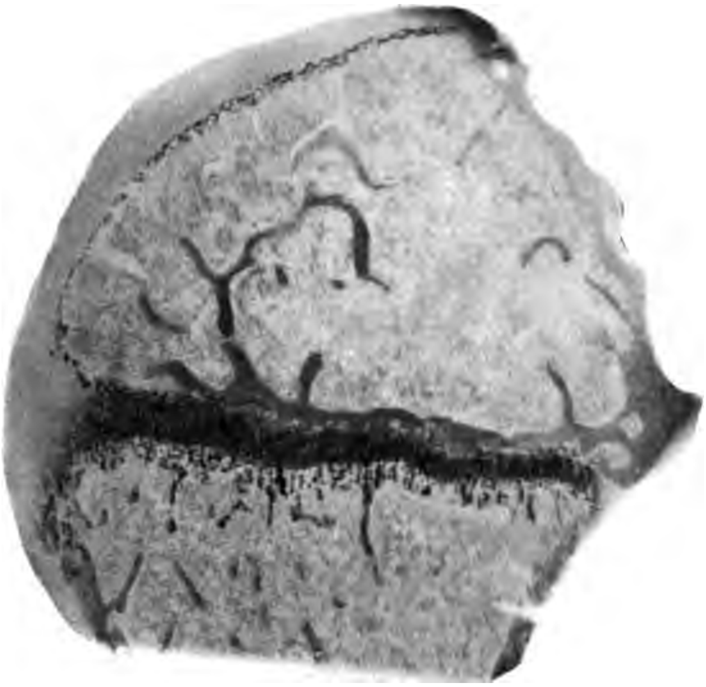

FIG. 8.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. VIII.)

PLATE 7.

FIG. 7. This picture shows the cartilage cells in the metaphysis during their transformation into osteoid. In it the fusion of the cells into osteoid tissue in the immediate vicinity of an invading blood vessel is well shown. (Diet of Lot 3133.)

FIG. 8. Section from the distal end of the femur of a rat (No. 819) on the diet of Lot 3137. In this diet 2 per cent of cod liver oil replaced 1.5 per cent of dextrin and 0.5 per cent of butter fat. This bone was in a condition of osteoporosis with essentially normal calcification. There is a provisional calcified zone and no metaphysis has been formed. Only physiological osteoid tissue is present.

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STUDIES ON EXPERIMENTAL RICKETS.

IX. LESIONS IN THE BONES OF RATS SUFFERING FROM UNCOMPLICATED BERI-BERI.

BY P. G. SHIPLEY,

(From the Department of Pediatrics, The Johns Hopkins University, Baltimore.)

AND E. V. McCOLLUM AND NINA SIMMONDS.

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore.)

PLATES 1 TO 4.

(Received for publication, October 11, 1921.)

As far as we are aware no investigators have reported observations on bone changes produced in animals by diets so constituted as to cause uncomplicated beri-beri or polyneuritis.

In studying the bones of rats suffering from polyneuritis induced by diets deficient in the water-soluble B we were surprised to find that in practically all histological details they were exactly like those of guinea pigs suffering from uncomplicated scurvy induced by a diet consisting of soy beans (cooked) 60.0, casein 10.0, wheat germ 10.0, maize 12.0, NaCl 1.0, $CaCO_3$ 2.0, butter fat 3.0, and cod liver oil 2.0 (Figs. 3 to 6). On this diet, which is complete except for the absence of the antiscorbutic substance, guinea pigs developed acute scurvy in about 3 weeks. When small amounts of orange juice or other antiscorbutic food are added to it they remain free from scurvy. These results were so unlooked for that we report preliminary observations on the bones of these rats. Further studies are in progress to determine the limits of dietary conditions which can induce such changes.

Polyneuritis was brought on in young rats by feeding a diet consisting essentially of purified food substances, and lacking (for the nutrition of the rat) only in the antineuritic substance, water-soluble B. The food mixture was constituted as follows:

Ration 3106.

	per cent
Casein	18.0
Salt mixture (185)	3.7
Agar-agar	2.0
Dextrin	71.3
Butter fat	5.0

On this diet the animals usually increased in weight during the first 3 weeks, then began to decline. After a few weeks (40 to 60 days) they usually developed the symptoms characteristic of beri-beri, although some animals died without developing the acute symptoms of the disease. (See Chart 1, Lot 3106 and Figs. 1 and 2.)

At autopsy all the body tissues of the rats were atrophic. There was no deformity of the skeleton and no noticeable enlargement of the extremities of the bones.

The picture of these bones through the microscope was quite indistinguishable from that seen in the bones of guinea pigs with scurvy (Figs. 3 and 4). The bones were very osteoporotic. One received the impression that all growth must have stopped. The epiphyseal cartilage was very shallow (only 4 to 5 cells in depth) and stained intensely with basic dyes. This was true of the cell bodies as well as the matrix. The cells in the narrow proliferative zone were very much flattened in the direction of the long axis of the bones. The intracellular substance of the cartilage was very abundant, and in the shallow zone of provisional calcification it was heavily infiltrated with lime salts. Here and there at intervals thick, heavily calcified rods of intracellular substance projected from the medullary border of the cartilage into the marrow cavity which was elsewhere in contact with the calcified provisional zone. Many of these projecting spicules of intracellular substance were rounded off and were capped by multinucleated masses of protoplasm which were apparently osteoclasts. The cortex of the shaft was of fair thickness and was solid and completely calcified. The marrow contained comparatively few nucleated cells (leucocytes and their predecessors). The blood vessels of the medullary cavity were crowded with red blood cells. In many places the vessels had ruptured and numerous small and large hemorrhages were to be found, especially in the vicinity

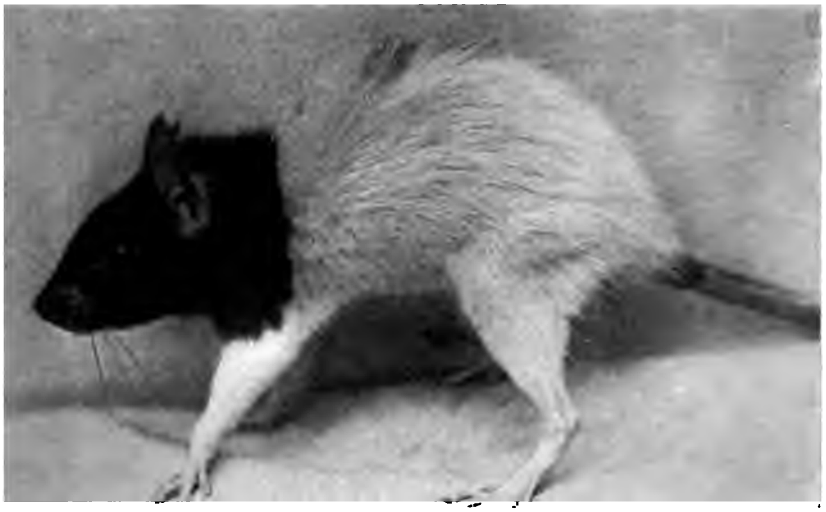

FIG. 1.

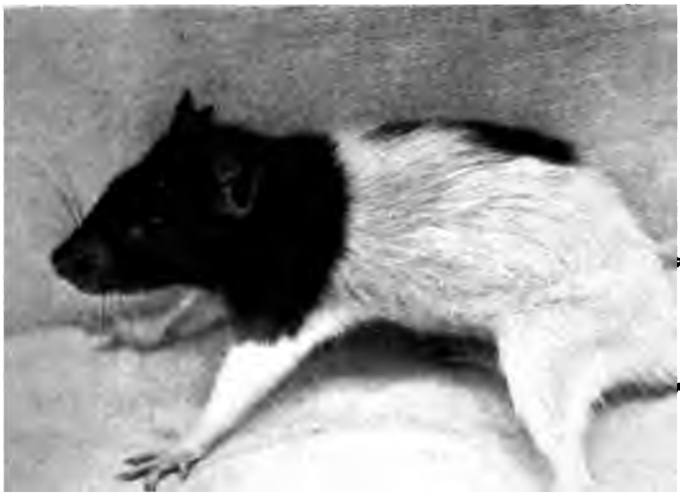

FIG. 2.

(Shipley, McCollum, and Simmonds: Studies on experimental rickets. IX.)

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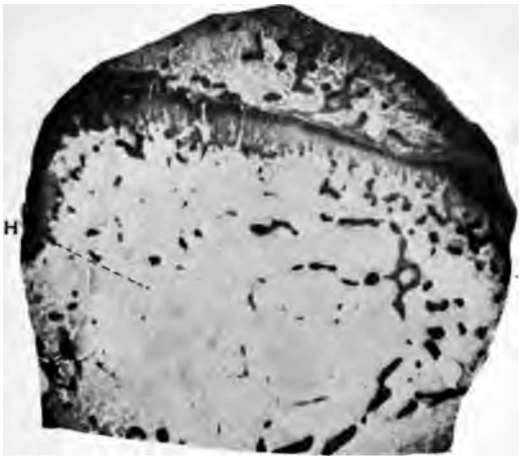

Fig. 3.

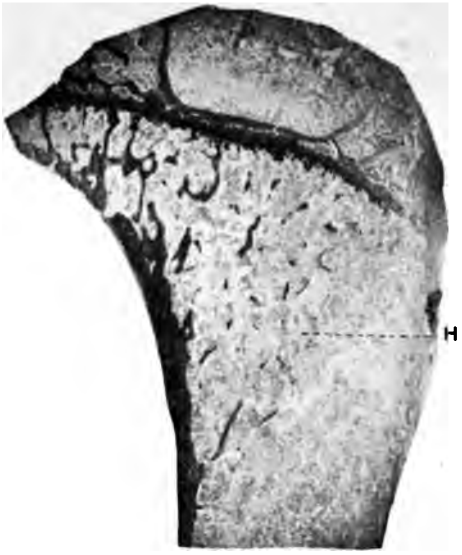

Fig. 4.

(Shipley, McCollum, and Simmonds: Studies on experimental rickets. IX.)

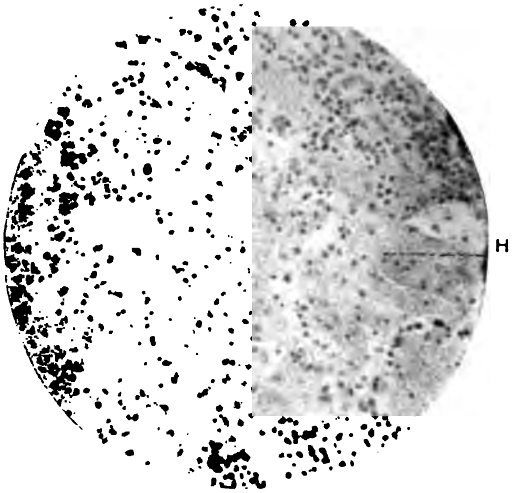

FIG. 5.

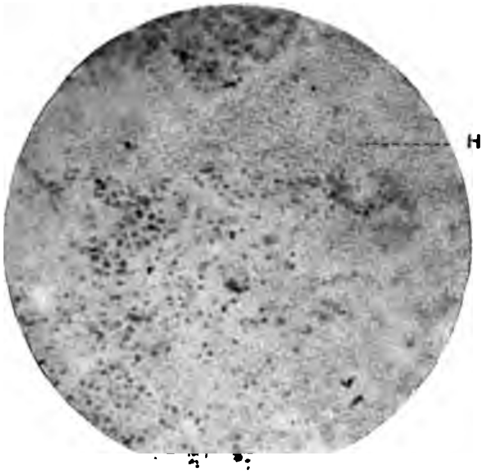

FIG. 6.

(Shipley, McCollum, and Simmonds: Studies on experimental rickets. IX.)

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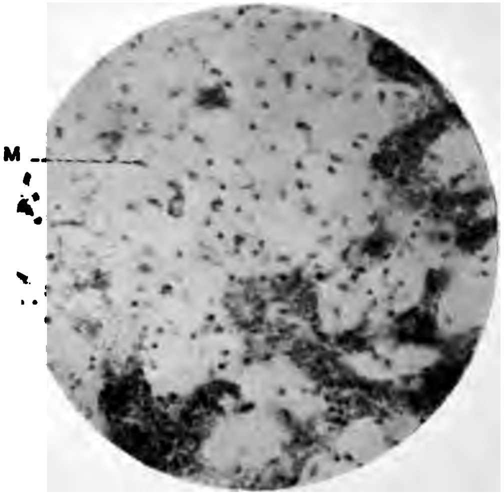

Fig. 7.

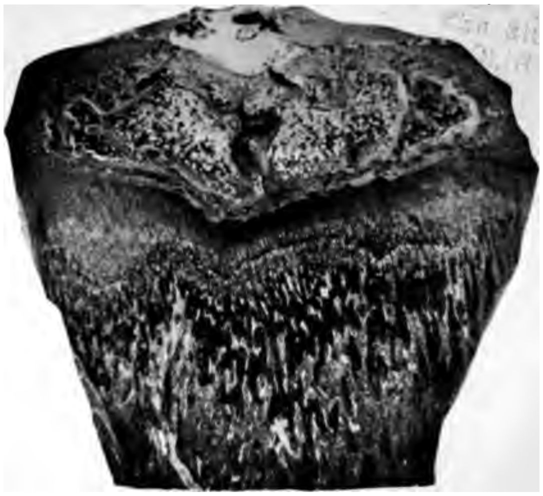

Fig. 8.

(Shipley, McCollum, and Simmonds: Studies on experimental rickets. IX.)

PUBLIC

of the cartilage (Fig. 5). In some bones the active bone marrow had been almost entirely replaced by these hemorrhages. Here and there at wide intervals small thick trabeculæ or trabecular remnants were to be found scattered through the hemorrhagic area which represents the marrow. These were completely calcified and were surrounded by an endosteum which consisted of a broken layer of osteoblasts, and a fine fibrillar network filled with mononuclear cells.

The destruction of the marrow elements is not the result of hemorrhage. The hematopoietic marrow may entirely disappear before hemorrhage has occurred and practically no trace of it remains except the supporting reticulum (Fig. 7). The medullary cavity under these conditions contains nothing but a cellular network of fine reticular tissue supporting numbers of thin walled blood vessels filled to capacity with corpuscles.

It may very well be that the dilatation and weakening of the blood vessels of the marrow and their consequent solution or rupture in the bones of rats on the diet low in water-soluble B is analogous to the condition of the intestine described by McCarrison (1). It is quite as likely that the vessel wall is directly affected by deprivation of water-soluble B as that the lesion is secondary to an affection of the nerve supply to the medullary vessels. The condition of the marrow is extremely interesting in the light of the findings of Happ,[1] who has studied the blood of rats in polyneuritis. He found that the number of red cells and the hemoglobin percentage was unchanged in the circulating blood. His animals showed, however, a marked leucopenia, with the white cells in one case as low as 1,800 per c. mm. (the normal white count for the rat is 10,000 cells per c. mm.). There was also a relative reduction in the number of polymorphonuclear cells and a marked shift to the right of Arneth's formula. This condition corresponds to the blood picture in human cases of beri-beri as reported by Findlay (2). Taken in conjunction with the condition of the bone marrow these findings can only indicate a markedly diminished hematopoiesis with abnormal conservation of the formed elements already in the circulation.

---

[1] Happ, W., personal communication. Dr. Happ's paper on the blood of rats which have been fed faulty diets is in press and will be published shortly.

Rats were fed on the same diet as the guinea pigs above described. Their growth is illustrated by the curves in Chart 2. Their bones were examined after they had been restricted to this scorbutic diet for 19 and 105 days. On this diet female rats not only grew to weights of 240 gm., but produced large litters of young (8, 10, and 12) and successfully weaned them. The young were not in an optimal condition but grew on the family diet for months and reached body weights of 200 gm. These results leave no room for doubt that the rat can dispense with water-soluble C provided the rest of the diet is satisfactory.

Examination of the bones of these rats revealed no evidence of departure from the normal in histological structure (Fig. 8).

The studies of McCollum and Simmonds some years ago (3) showed that rats may subsist and grow well for many months on diets free from the antiscorbutic substance, and show no evidence of developing scurvy. Parsons (4) showed that livers of rats long deprived of the antiscorbutic factor will promptly cure scorbutic guinea pigs. The prairie dog has also been shown to be capable of growth and long maintenance on a diet which will promptly induce scurvy in guinea pigs (5).

The growth and state of nutrition of another group of rats restricted to this food mixture (Lot 2983, Chart 1) supplemented with 30 per cent of rolled oats which served to supply an abundance of water-soluble B also show that there was no known defect in our diet for the rat other than lack of the dietary factor responsible for beri-beri; *i.e.*, water-soluble B, Chart 1 (6). Oats contain neither fat-soluble A nor water-soluble C in demonstrable amounts. This purified food mixture supplemented with 3 per cent of wheat germ, or with an extract of wheat germ, is likewise capable of inducing growth (see Chart 3). A similar mixture of purified foodstuffs (with butter fat) and supplemented with 25 per cent of cooked navy beans also induces good growth and fertility in the rat (see Chart 4). It will be evident, therefore, that the rats on Formula 3106 were fed a diet which was complete for the rat except for the lack of antiberi-beri substance, water-soluble B.

The bones of rats fed the same diet with 30 per cent of rolled oats were studied histologically. These revealed no lesions whatever, notwithstanding the specific fasting of these rats for the antiscorbutic substance, water-soluble C. Tozer (7) claimed in a foot-note to a paper describing the lesions in the bones of scorbutic guinea pigs that a deficiency of the fat-soluble A in the diets of these animals may cause changes "indistinguishable from those termed incipient and definite scurvy." The bones of rats on diets low in this substance alone are osteoporotic (Chart 1, Lot 2031). They have no changes resembling the lesions of scurvy. The bone marrow is quite normal and no hemorrhage or congestion is present.

CONCLUSIONS.

1. Rats fed a diet complete except for the absence of the anti-beri-beri factor develop lesions in the bones which are essentially identical with those seen in guinea pigs suffering from acute and uncomplicated scurvy. Rats confined to the same diet supplemented with water-soluble B do not show these changes.

2. The bones of rats on a diet which is only deficient in the fat-soluble A are osteoporotic but have no other resemblance to the bones of scorbutic animals.

CHART 1, Lot 3106. These rats were restricted to a diet which was adequate in all respects except that it lacked entirely the antineuritic substance, water-soluble B. The animals were unable to grow because of this deficiency and in many cases developed the typical symptoms of polyneuritis.

The bones of these animals were not deformed as in rickets, but were osteoporotic and exhibited characteristic hemorrhages under the micro-cope. These peculiarities have been described in the text of this paper. They were indistinguishable from the bones of guinea pigs which were uffering from acute scurvy.

Lot 2983 had a diet essentially similar in all its properties to that of the nimals just described (Lot 3106) except that the *antineuritic substance* was added in the form of 30 per cent of rolled oats. Oats contain no anti-corbutic factor and no demonstrable amount of fat-soluble A. The latter actor was furnished to both groups (Lots 2983 and 3106) in the form of but-er fat. While the animals did not grow at the maximum rate, nor reach he maximum adult size, they were fairly well nourished and were fertile. They destroyed almost all their young soon after they were born.

This diet, entirely free from antiscorbutic substance, but containing he antineuritic factor, induced the development of entirely normal bones. There seems to be no other interpretation warranted than that the specific one changes found in Lot 3106 were due to lack of water-soluble B in the diet.

Lot 2031 is included here for the sake of completeness. It contained in he rolled oats a sufficient amount of water-soluble B, as is shown by the growth and reproduction of Lot 2983. It was entirely deficient in the

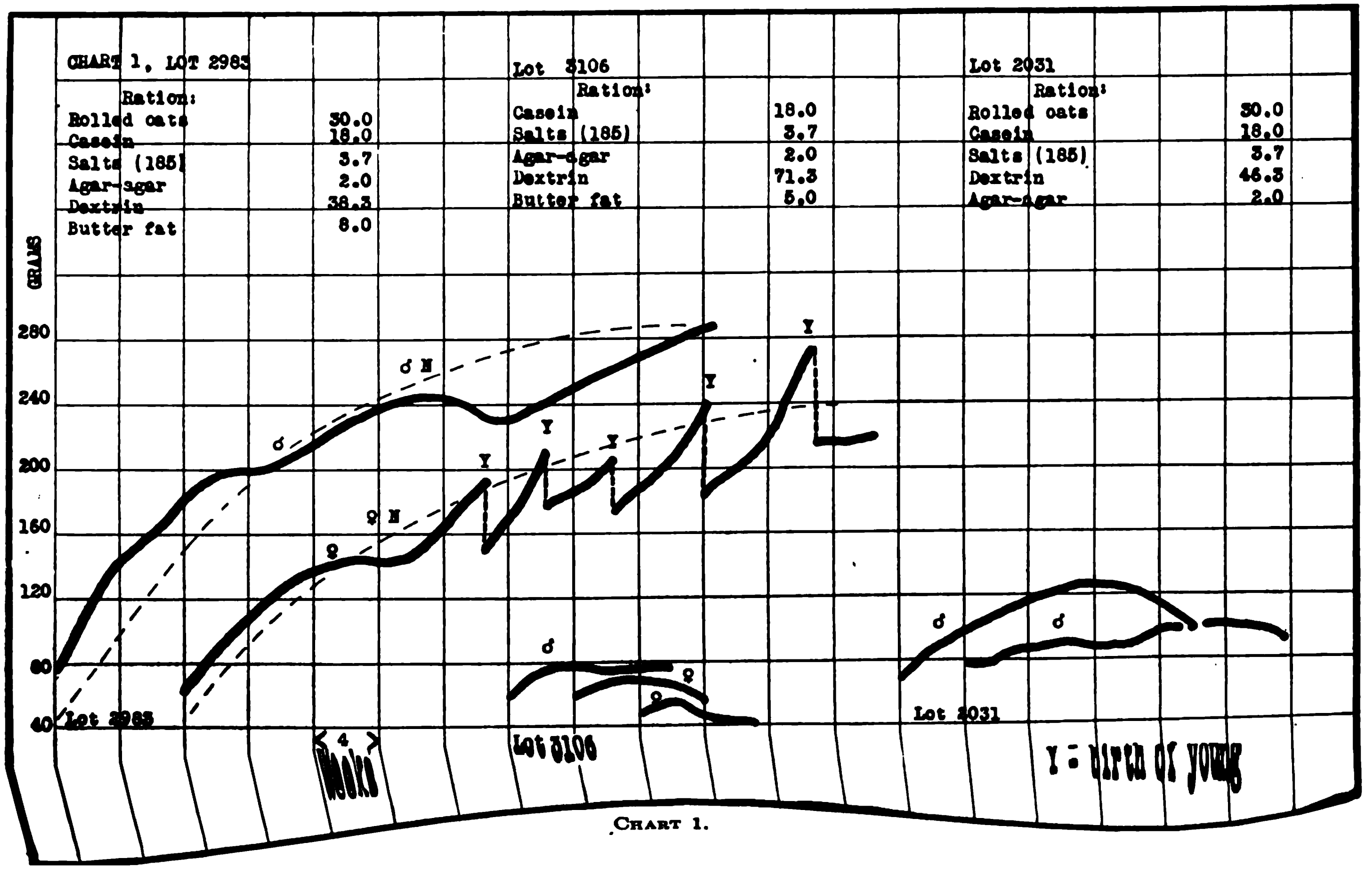
CHART 1, LOT 2983
Ration:
Rolled oats 30.0
Casein 18.0
Salts (185) 3.7
Agar-agar 2.0
Dextrin 38.3
Butter fat 8.0
Lot 3106
Ration:
Casein 18.0
Salts (185) 3.7
Agar-agar 2.0
Dextrin 71.3
Butter fat 5.0
Lot 2031
Ration:
Rolled oats 30.0
Casein 18.0
Salts (185) 3.7
Dextrin 46.3
Agar-agar 2.0
GRAMS
280
240
200
160
120
80
40
♂ N
♂
♀ N
♀
Y
♂
♀
Lot 2983
4
Weeks
Lot 3106
Lot 2031
Y = birth of young

CHART 1.

antiscorbutic substance and, because of the omission of butter fat from the formula, the diet was essentially free from fat-soluble A. Since all the evidence supports the view that the antiscorbutic substance is dispensable from the diet of the rat the diet of this group was faulty only in respect to lack of fat-soluble A. The addition of butter fat (fat-soluble A) makes the food mixture like that of Lot 2983, which was complete.

Lack of fat-soluble A did not in these experiments induce changes in the bones which in any way resembled those described and attributed in the rat to beri-beri and in the guinea pig to scurvy. The bones were osteoporotic (8). With the addition of butter fat the diet became capable of supporting normal bone development.

We conclude from these studies that *in the rat* restricted to a diet of purified food substances (free from all vitamines) the addition of fat-soluble A alone induces bone changes identical with those seen in scurvy in guinea pigs, whereas the addition of water-soluble B (in the absence of fat-soluble A) induces osteoporosis. It is further to be noted in agreement with earlier studies that diets containing satisfactory amounts of calcium and phosphorus do not produce rickets even when nearly free from fat-soluble A (8).

CHART 2, Lot 3168. These rats were restricted throughout the entire growth period and later to a diet which induces severe scurvy in the guinea pig within 2 to 4 weeks. Rats are, however, able to grow rapidly to the full adult size, exhibit the normal fertility, and rear their young with a low mortality rate. The second generation was able to grow to maturity and reproduce and rear young. There can be no question that the rat may be satisfactorily nourished without a supply of the antiscorbutic substance, water-soluble C.

The histological picture of bone sections from these animals showed the normal structure.

CHART 3, Lot 2089. This diet was essentially comparable to that of Lot 3106 (Chart 1) except that it contained water-soluble B derived from the alcoholic extract of 10 gm. of wheat germ per 100 gm. of ration. This induced essentially normal growth, although the animals remained somewhat undersized. These animals were produced some years ago in our laboratory and their bones were not examined. We know, however, that rats which were able to grow as well as these did would have bones with no serious defects.

Lot 2088 was fed a diet of purified food substances completed qualitatively by the addition of fat-soluble A and water-soluble B in 4 per cent of butter fat and 3 per cent of wheat germ respectively. Fair growth was possible and one female had three litters of young.

CHART 4, Lot 696. These rats were fed a diet derived in great measure of purified food substances, but supplemented with water-soluble B in the form of 25 per cent of cooked navy beans, and fat-soluble A in 5 per cent of butter fat. This diet contained no antiscorbutic substance, yet young rats were able to grow rapidly to full adult size when restricted to it. Even the second generation of rats on this diet grew fairly well.

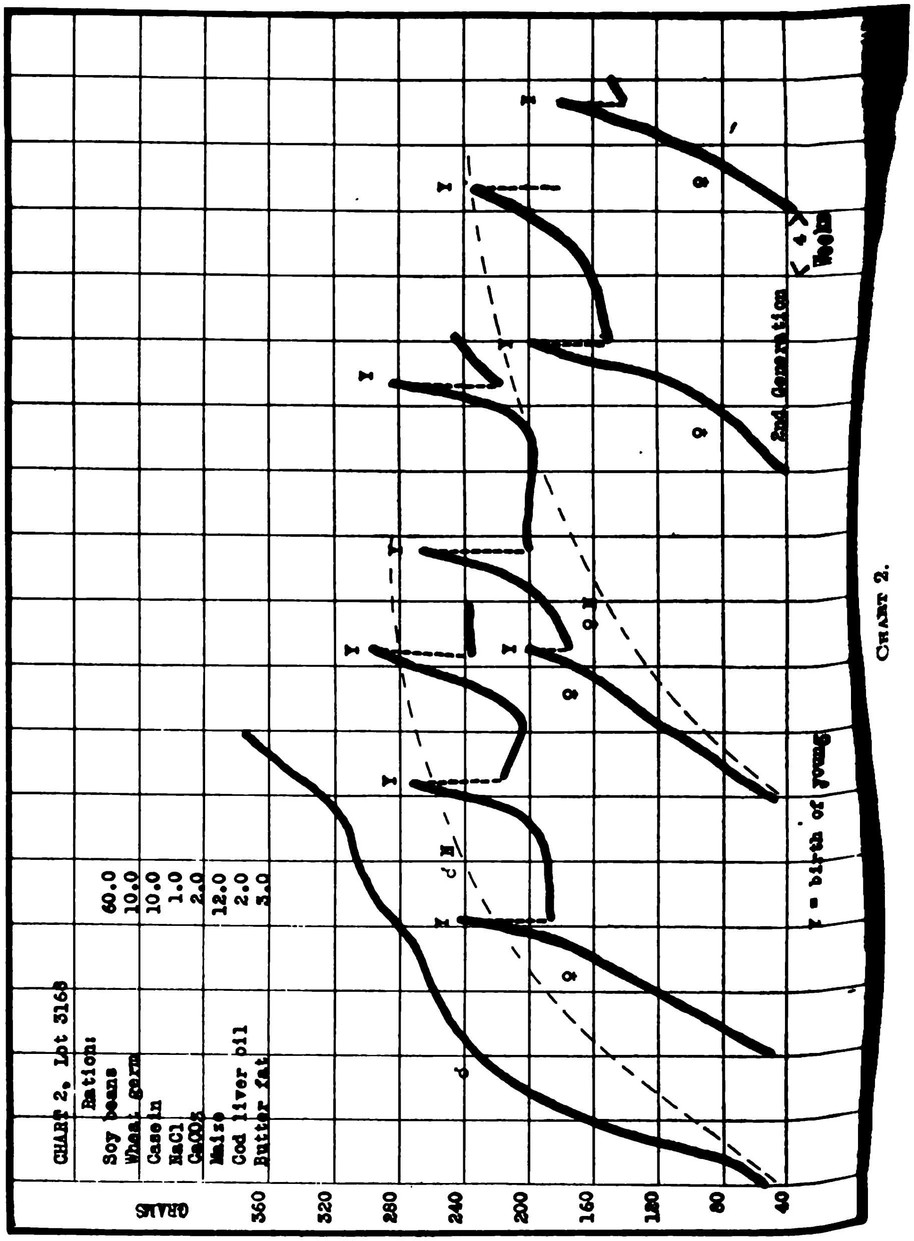
CHART 2. Lot 3168
Ration:
Soy beans 60.0
Wheat germ 10.0
Casein 10.0
NaCl 1.0
CaCO3 2.0
Maize 12.0
Cod liver oil 2.0
Butter fat 3.0
GRAMS
360
320
280
240
200
160
120
80
40
X
♂
♀
♂ M
♀ M
2nd Generation
4 Weeks
X = birth of young

CHART 2.

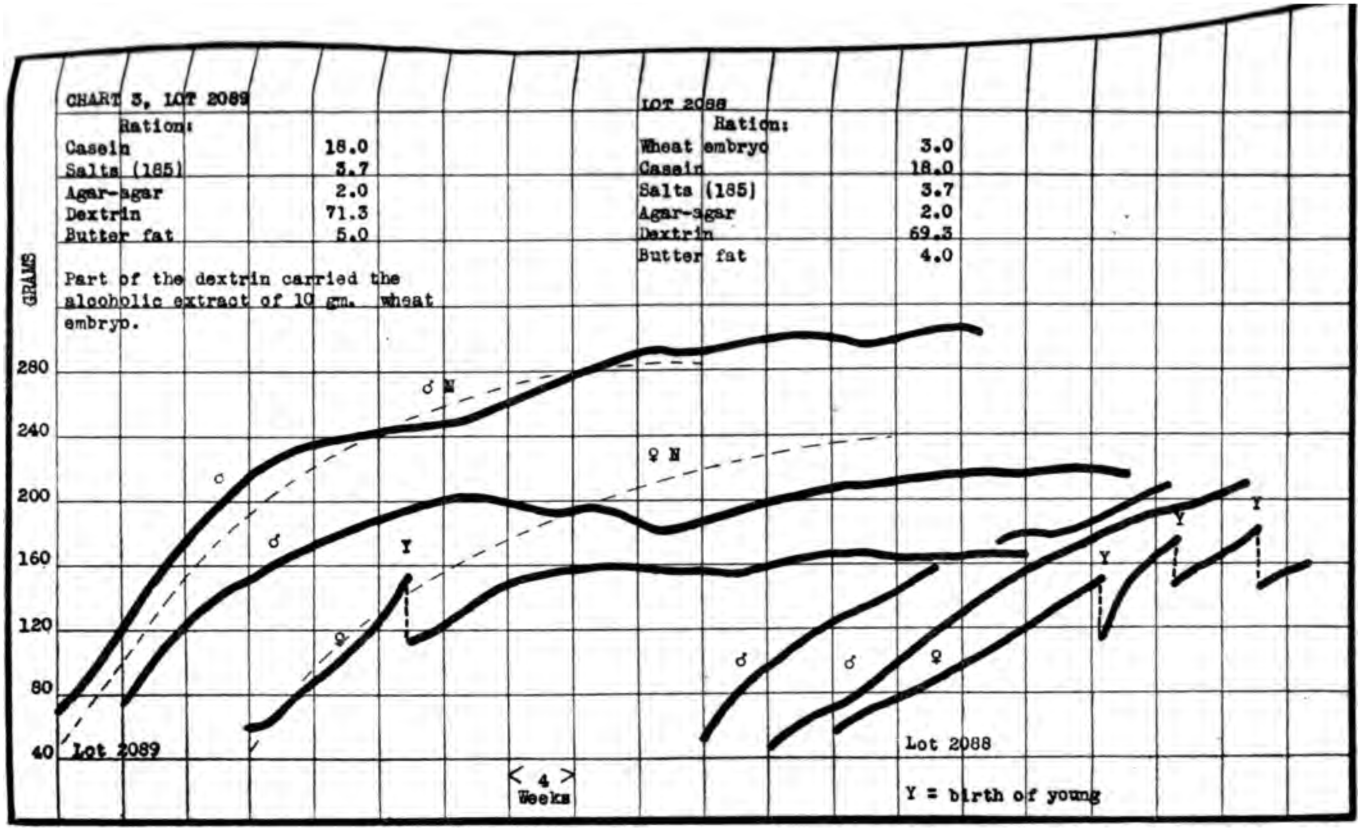
CHART 3, LOT 2089
Ration:
Casein 18.0
Salts (185) 3.7
Agar-agar 2.0
Dextrin 71.3
Butter fat 5.0
Part of the dextrin carried the alcoholic extract of 10 gm. wheat embryo.
LOT 2088
Ration:
Wheat embryo 3.0
Casein 18.0
Salts (185) 3.7
Agar-agar 2.0
Dextrin 69.3
Butter fat 4.0
GRAMS
280
240
200
160
120
80
40
♂ N
♀ N
♂
♂
♀
Y
Y
Y
Y
♂
♂
♀
Lot 2089
Lot 2088
< 4 >
Weeks
Y = birth of young

CHART 3.

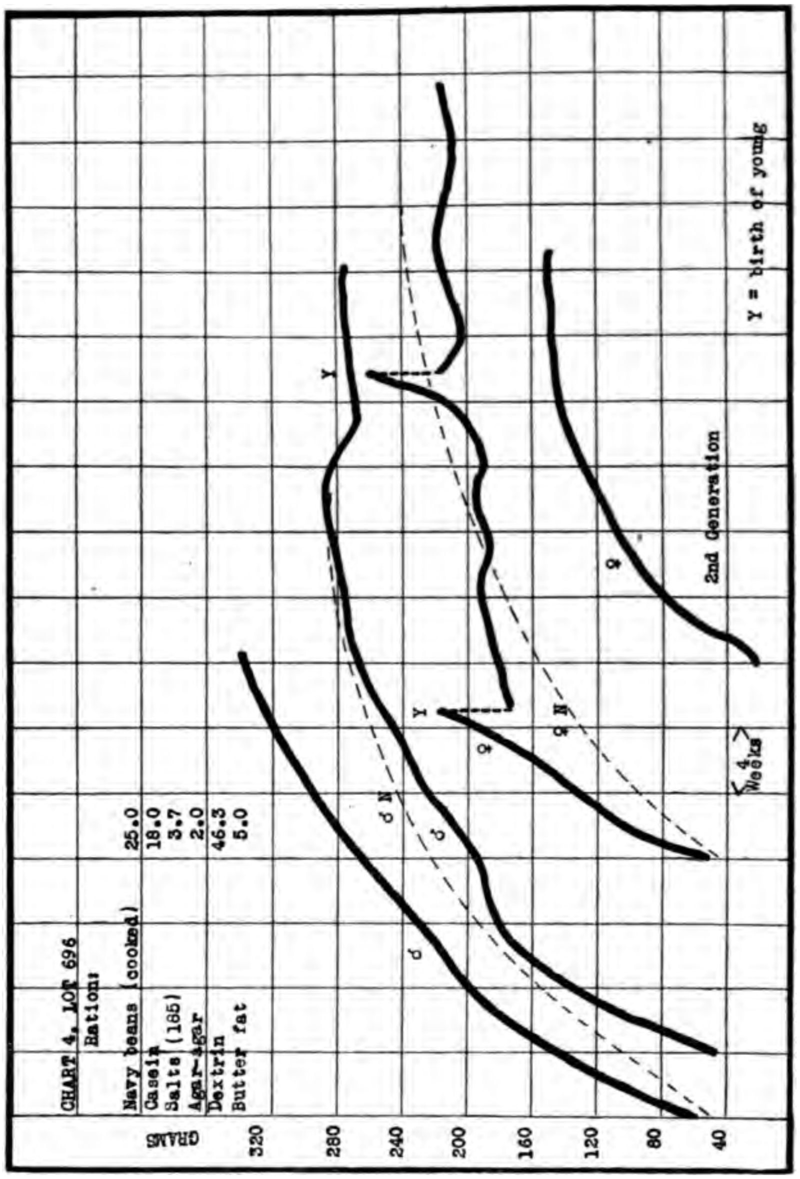
CHART 4. LOT 696
Ration:
Navy beans (cooked) 25.0
Casein 18.0
Salts (185) 3.7
Agar-agar 2.0
Dextrin 46.3
Butter fat 5.0
GRAMS
320
280
240
200
160
120
80
40
♂
♂ N
♂
Y
♀
♀ N
Y
♀
2nd Generation
Y = birth of young
4 Weeks

CHART 4.

These several groups of growth curves of rats are presented in order to illustrate how effectively a diet of purified food substances can be supplemented by means of additions which do not contain demonstrable amounts of the antiscorbutic substance. They form a complete demonstration of the fact that the bone changes induced in those rats which were selectively fasted for water-soluble B only were due to lack of this dietary essential.

It has been pointed out by Vedder (9), Hess (10), and others, that there are similar nervous manifestations and pathological changes in the heart in human cases of scurvy and beri-beri. Unfortunately, one cannot place much confidence in the description of these diseases as they occur in man, for an examination of the diets on which people develop either of these diseases makes it practically certain that beri-beri scarcely ever occurs except in an individual who is a border-line case of scurvy. Scurvy doubtless has, however, occurred many times without complication with beri-beri. We have no assurance that any description of histological studies of any human tissues were made on subjects suffering from one of these diseases uncomplicated with the other.

Nevertheless there is an added interest, in the repeated statements of others that there are lesions in beri-beri and scurvy which are common to the two diseases. Our observations show that the histological changes in the bones of rats suffering from uncomplicated beri-beri are apparently identical with those seen in the guinea pig suffering from uncomplicated scurvy.

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EXPLANATION OF PLATES.

Plate 1.

Figs. 1 and 2. This rat was in a condition of polyneuritis induced by feeding Diet 3106 for 70 days. Fig. 1 shows the animal rising to his feet from the spread-eagle posture assumed during rest in a ventral position (Fig. 2). Note the spasticity and the extreme extension and abduction of all four extremities, the extension of the digits, and the roughness of the coat.

Plate 2.

Fig. 3. Section of a long bone from a guinea pig with acute scurvy. The bone is well calcified throughout but is extremely osteoporotic. The marrow cavity shows numerous hemorrhages. *H* = areas of hemorrhage. Leitz microsummar. 35 mm. objective. No ocular.

Fig. 4. Long bone of a rat killed during an attack of polyneuritis. This bone was osteoporotic and the hematopoietic marrow was almost entirely replaced by hemorrhage. *H* = areas of hemorrhage. Same magnification as Fig. 3.

Plate 3.

Fig. 5. High power picture of the marrow cavity of the bone shown in Fig. 3 to show the hemorrhagic marrow. *H* = areas of hemorrhage. Leitz objective No. 6. No ocular.

Fig. 6. To show the replacement of the bone marrow by hemorrhages during polyneuritis. *H* = hemorrhage. Magnification as in Fig. 5.

Plate 4.

Fig. 7. Bone marrow of a polyneuritic animal during the congestion stage before hemorrhage has occurred. The marrow consists only of reticular tissue supporting widely dilated congested blood vessels. *M* = marrow. Same magnification as in Fig. 5.

Fig. 8. To show the bone of a rat which was fed on the diet which we used to induce scurvy in our guinea pigs. This bone is quite normal.

Studies on Experimental Rickets.

Rickets and Rickets-like Disease Produced in Rats by Deficient Diets.

By Dr. P. G. SHIPLEY and Dr. E. A. PARK

(*From the Department of Pediatrics, The Johns Hopkins University, Baltimore, Md.*)

AND

Dr. E. V. McCOLLUM and NINA SIMMONDS

(*rom the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.*)

(Reprinted from the DENTAL COSMOS for March 1922.)

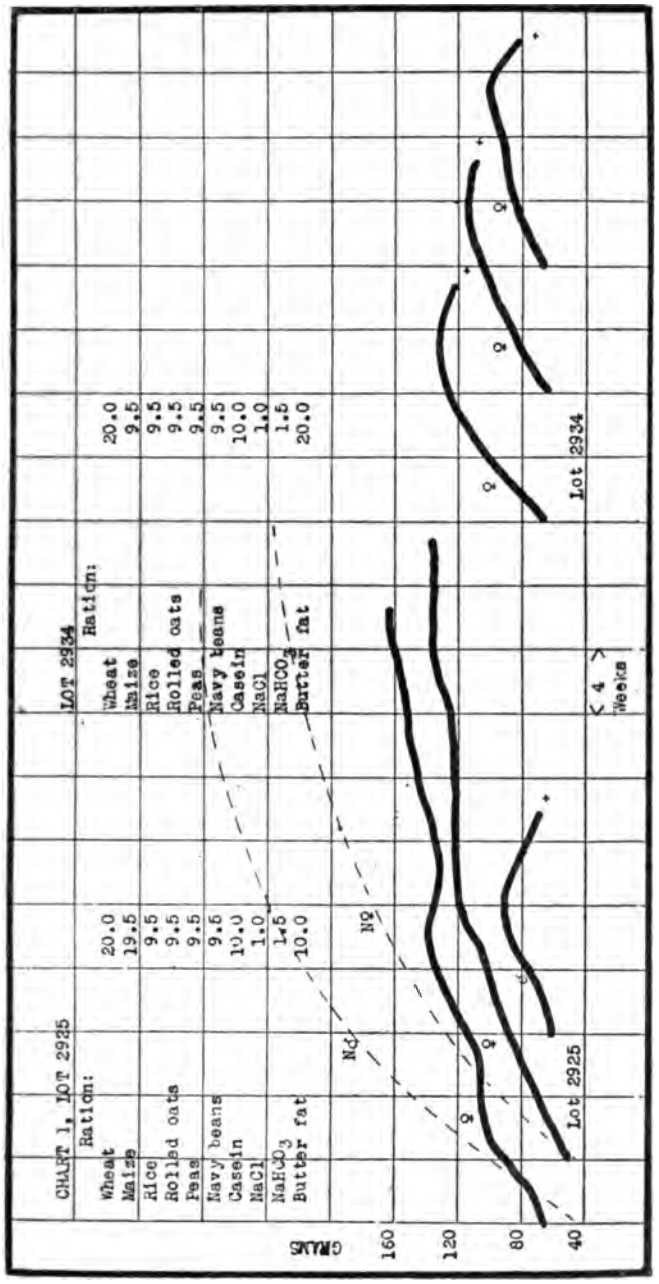


CHART 1, LOT 2925
Ration:
Wheat 20.0
Maize 19.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Casein 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 10.0
LOT 2934
Ration:
Wheat 20.0
Maize 9.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 9.5
Casein 10.0
NaCl 1.0
$NaHCO_3$ 1.5
Butter fat 20.0
GRAMS
160
120
80
40
N♂
N♀
♀
♀
♀
♀
♀
Lot 2925
Lot 2934
< 4 >
Weeks

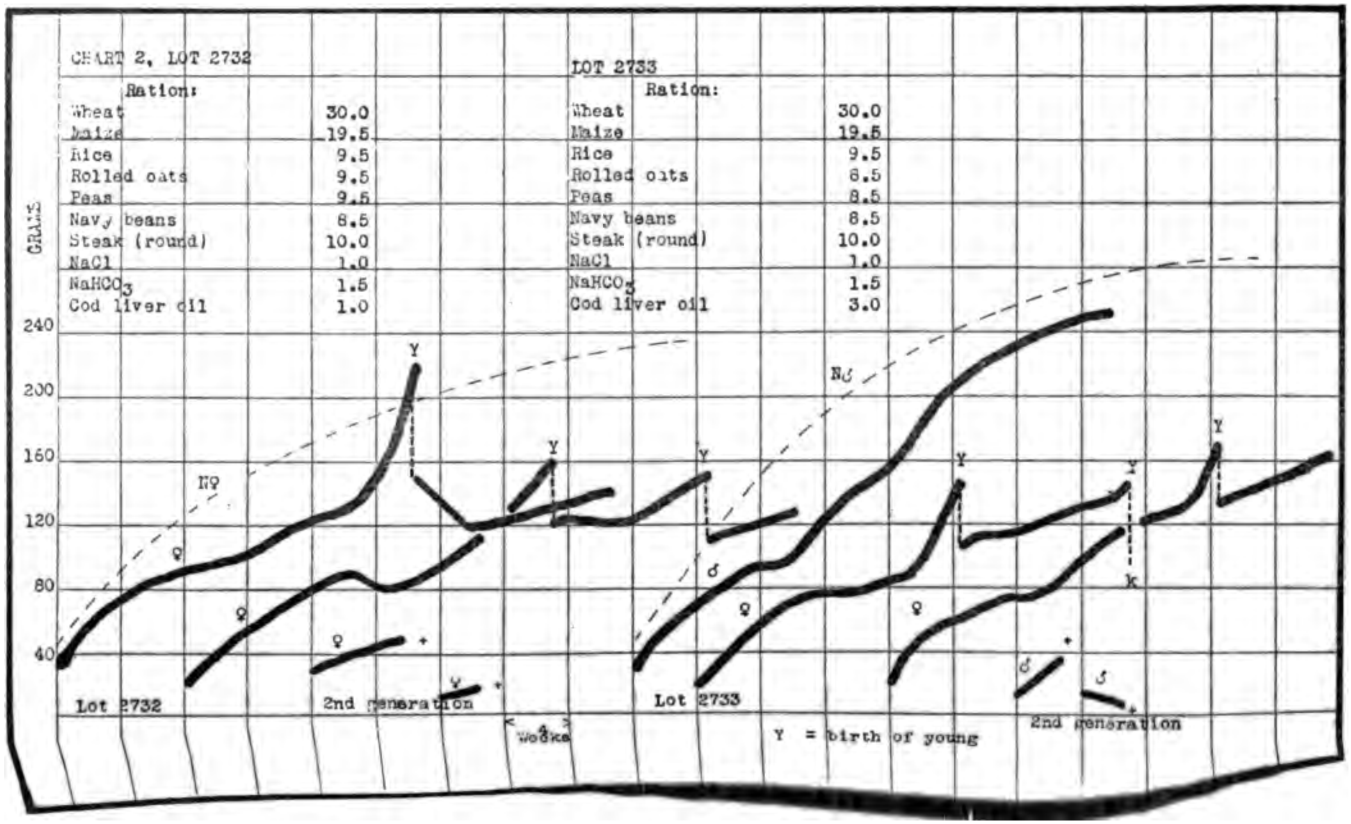


CHART 2, LOT 2732
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 9.5
Peas 9.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
NaHCO3 1.5
Cod liver oil 1.0
LOT 2733
Ration:
Wheat 30.0
Maize 19.5
Rice 9.5
Rolled oats 6.5
Peas 8.5
Navy beans 8.5
Steak (round) 10.0
NaCl 1.0
NaHCO3 1.5
Cod liver oil 3.0
GRAMS
240
200
160
120
80
40
N♀
N♂
♀
♂
Y
k
+
Lot 2732
2nd generation
< 4 weeks >
Lot 2733
Y = birth of young
2nd generation

Cod liver oil, we have repeatedly observed with different diets, protects growing rats against the injurious effects of lack of calcium and enables them to grow and appear well nourished for a considerable period, where they would fail to grow and would be very inferior with even much greater amounts of butter fat instead of the cod liver oil.

It is especially remarkable that whereas 3 per cent of butter fat suffices when added to food mixtures of the type under discussion to meet all the requirements of the rat for fat-soluble A and for the specific calcium-depositing factor, if there be one, when the diet contains favorable concentrations of calcium, 10 to 20 per cent of butter fat fails to shield them in a manner at all comparable with 1 per cent of cod liver oil. Again, it is remarkable that animals specifically fasted for calcium are protected as effectively by 1 per cent of cod liver oil as by 3 per cent or higher planes of intake.

We do not believe that our samples of butter fat could differ in the concentration of the calcium-depositing substance, since, as will be shown in later charts, when the diet contains calcium in amounts ranging from 0.1 to 0.5 per cent of the carbonate, butter fat becomes adequate to supply the nutritive needs of the body and the differences in the effects of cod liver oil and of butter fat in the diet tend to disappear.

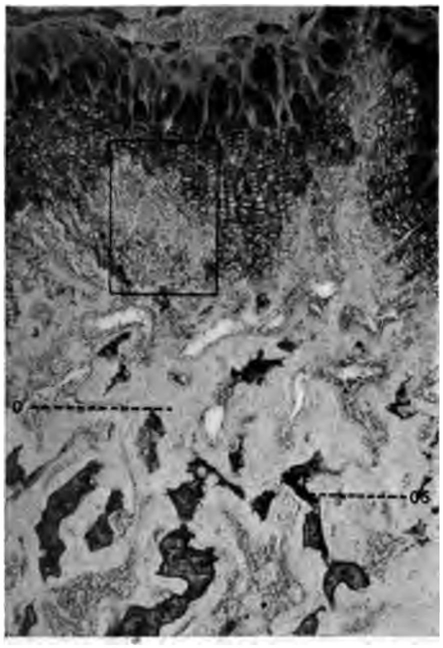
CHART 3. Lot 2821 was fed a diet essentially comparable to those discussed in the preceding charts except that its content of 20 per cent of casein raised the phosphorus content of the food mixture. Like the preceding diets it was very poor in calcium. 5 per cent of butter fat did not suffice to protect the animals against lack of calcium. This chart should be compared with Chart 4, Lot 2822, which differed significantly only in containing 2 per cent of cod liver oil and 3 per cent of butter fat.

Lot 2821 was very poorly nourished and became badly deformed. The coats of these rats were rough and thin (see Fig. 1) and they aged very early.

CHART 4. Lot 2822 had a diet exactly similar to that of Lot 2821 (Chart 3) except that 2 per cent of cod liver oil replaced 2 per cent of butter fat. This modification of the diet made a remarkable difference in their growth and well being (see Fig. 2). The former were stunted, infertile, and short lived. The latter grew to full normal size, presented a well nourished appearance, and were fairly fertile, and succeeded in rearing a considerable number of their young to the weaning age. The young were puny, pot-bellied, almost completely stunted in growth, and died early. The mothers declined rapidly after nursing two or three litters. The males, while well nourished for an interval following the completion of growth, soon presented a poorly nourished appearance and aged early.

The effect of the cod liver oil was to make the animals in some degree immune for a time to the injurious effects of lack of calcium. Even ten times as much butter fat could not do this. If a small addition of calcium were made to this diet the butter fat would supply sufficient of some organic ceives a deposit of calcium and becomes true bone. Calcification of the pre-osseous tissue is very rapid during the formation of normal bone, so that only a very narrow border (1 to 11μ) of osteoid tissue can be found about bone trabeculæ in normal young animals. This is known as the physiological osteoid.

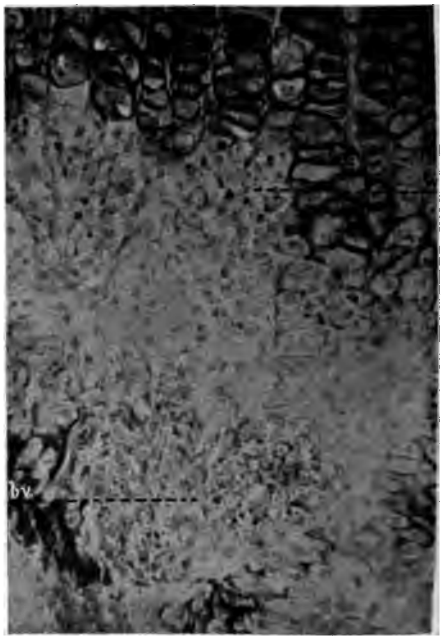
FIG. 2.



Photomicrograph of an area, corresponding to that shown in Fig 1, from a rachitic bone. Note the irregularity of the cartilage and the tongue-like projections of that tissue into the shaft. The cartilage is ragged, is not calcified and is invaded by bloodvessels. The trabeculæ are crooked and very thick, and the small amount of calcified bone (*os*) is surrounded by a broad zone of osteoid tissue (*o*).

In rachitic bones a provisional zone of calcification is not formed, since the organism fails to deposit lime salts in the intercellular substance of the cartilage. The proliferative cartilage persists in some places, and in others is destroyed in an irregular way by aberrant capillaries, which sprout in a disorderly fashion from the parent vessels in the shaft and invade the cartilage in all directions (Figs. 2 and 3). Thus the line of junction of the epiphysis and the shaft becomes very irregular and long tongues and islands of cartilage are left isolated in the metaphysis and diaphysis. At the same time bone is not formed normally either as regards the direction of the trabeculæ, since there is no calcified intercellular substance to serve as a guide for the activity of the osteoblasts or as regards ossification, since calcium salts are not deposited in the osteoid tissue (Fig. 2). Osteoid is formed in excessive amounts, perhaps as an attempt at compensation for the loss of strength

FIG. 3.

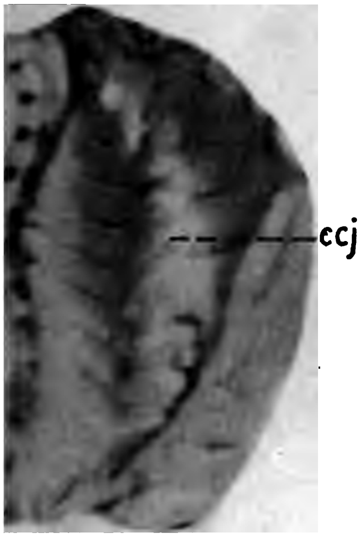


High-power photomicrograph of the area marked in Fig. 2, to show the bloodvessels (*bv*) destroying areas of cartilage. This picture also shows the transformation of cartilage cells into osteoid tissue.

bone from failure of the pre- tissue to calcify.

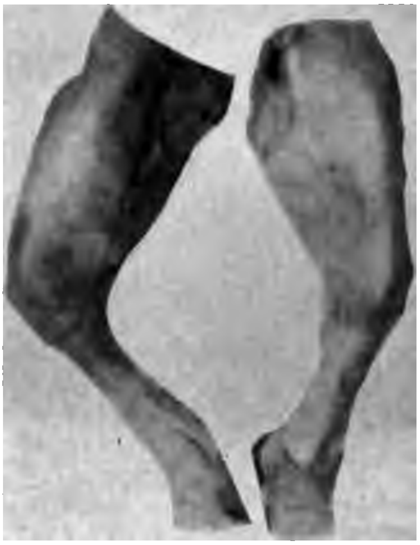
iis way it comes about that the ; region of the bone becomes an ally wide area made up of blood- isolated masses and tongues of e, connective tissue and osteoid ed rachitic metaphysis). All lefects, with the exception of the of the provisional zone of calcifi- are perversions or exaggerations nal physiological processes which all through the life of a healthy According to Schmorl the rachitic must be regarded as the result verproduction of pre-osseous tissue fails to receive a deposit of lime nd not of the removal of calcium from bone formed and calcified before the onset of the disease.

FIG. 4.



he pleural surface of the left thorax achitic rat. This rat had a rachitic sis. The junctions of the ribs with cartilages form an angle represented eep groove on the lateral surface of the wall. This angle (*ccj*) forms two grooves on the pleural surface and its divides the thorax into two chambers.

FIG. 5.

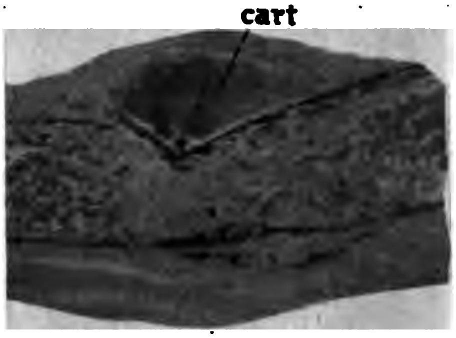


Bow-legs from a rachitic rat.

The dietary factors chiefly concerned in the production of the lesions which have been induced in the bones of the animals studied by us are calcium, phosphorus and an organic dietary constituent which may or may not be identical with the anti-xerophthalmic fat-soluble A. We have found the production of pathological osteoid tissue to follow the administration of a large variety of deficient diets. Rations which are low in calcium alone or in calcium and the fat-soluble A fail to furnish the conditions necessary for the normal genesis of bone when the deficiency is uncomplicated. The bones of animals maintained on diets low in calcium salts are found to show a characteristic lesion, which has certain fundamental resemblances to human rickets, but differs from that disease as it is usually seen in man in details which have been reported in full in another publication.[9]

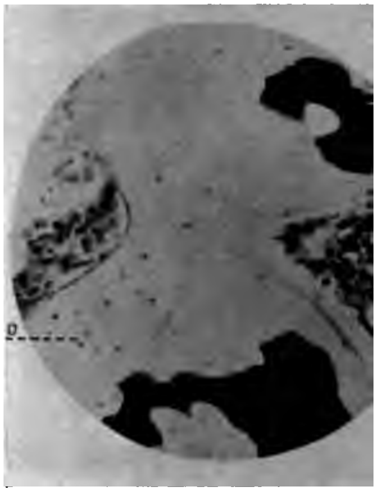
Diets which are deficient only in the organic substance never result in rickets but in a condition of osteoporosis with complete calcification of all bony tissue. If, however, there is also a deficiency of phosphate from such a diet, the calcium intake being maintained at approximately the optimal level for the growth of the animal, a disease is produced which has fundamental characteristics in common with rickets as it occurs in man, especially with the lesions of the so-called healing rickets. The condition is marked by failure of production of the provisional zone of calcification, great overproduction of osteoid tissue, and a very marked degree of persistence of the cartilage cells in the shaft, where they undergo transformation directly into osteoblasts. They aid in the building up of osteoid tissue. Animals maintained on such a diet show a marked tendency to return to normal and the bones show all grades of transition from severe rickets to a condition of osteoporosis (Fig. 5). If diets which are low in the organic substance and in phosphate are supplemented with a complete salt mixture which contains an adequate amount of phosphate, rickets never develops but keratomalacia (xerophthalmia) does. The bones of such rats ar oporotic. Microscopic examinat the bones of rats shows that certai deficient in both the lime salts a organic substance, but containi proximately optimal amounts of phate, result in the productior lesion closely related to if not ic with rickets. There is compe

FIG. 6.



Photomicrograph of a section through a spontaneous green-stick fracture of a rib. The site of the fracture is marked by the nodule of cartilage (*cart.*), the appearance of which precedes the formation of an osteoid callus.

FIG. 7.



The calcified portions of the trabecu are surrounded in rachitic bones b zones of osteoid (*o*); *m*, marrow.

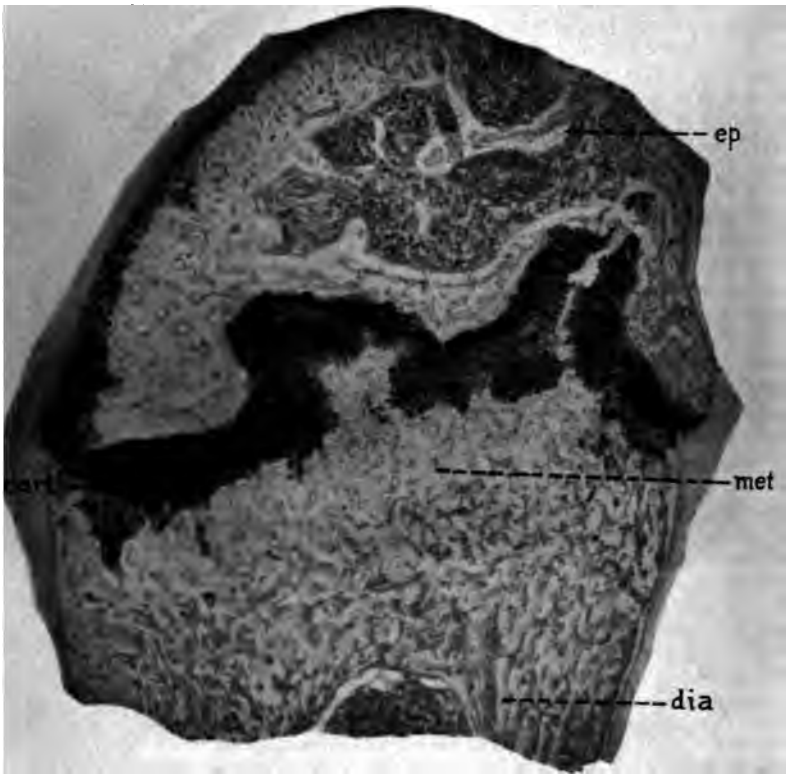
overproduction of osteoid which relation to absorption of lime sal irregular invasion of the cartilag the persistence of its cells. Th liferative cartilage remains unca a metaphysis is formed of blood connective tissue and osteoid, a cartilage isolated in the metaph the growing bone (Figs. 2, 3 and

Another type of diet which pr rickets in the rat may be represen the following formula:

Wheat,	33.0
Maize,	33.0
Wheat gluten,	15.0
Gelatin,	15.0
NaCl,	1.0
$CaCO_3$,	3.0

a diet is low in phosphate and a very small amount of the substance. On the other hand, lant amount of calcium is presie food (Figs. 4, 5, 6, 7 and 9). ıchitic process is most severe in rapidly growing bones, *i e.,* the lower end of the femur and the : end of the tibia, and in the ıidly growing animals.

FIG. 8.



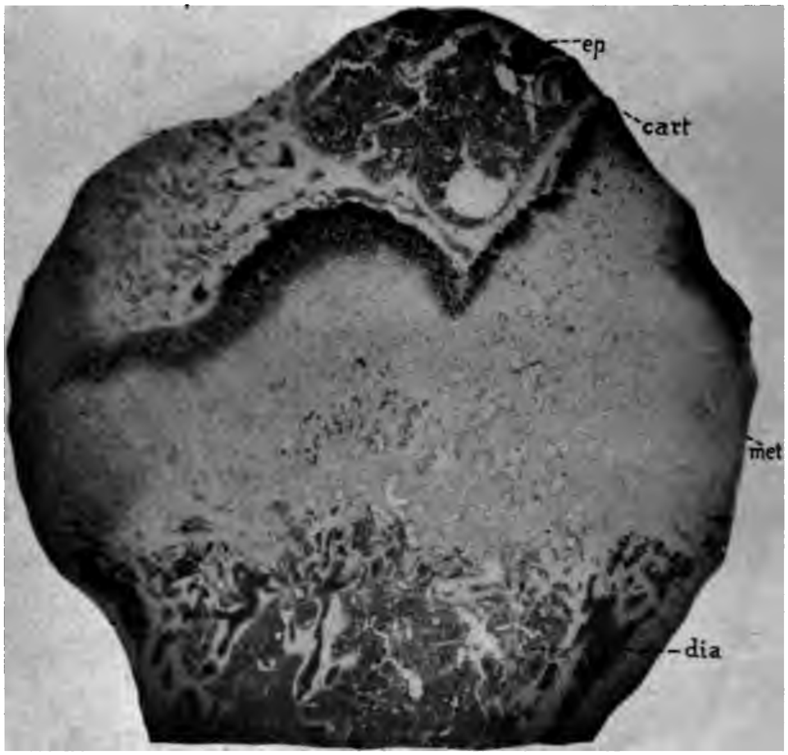
ɔf the rat from which this rachitic bone was taken was that of lot No. 3143, which ins an abundant supply of calcium salts, but is deficient in the organic substance n phosphate.

Legend

ıg will occur even in an animal take of lime salts is much below ıal under the influence of cod- , with the re-formation of the ıal calcified zone and calcification of the metaphysis. Cod-liver oil is much more potent than butter fat to induce healing in rachitic bones or to protect an animal against the disease. The first evidence of healing rickets in the rat, as in man, is the deposition of calcium in the proliferative cartilage on the chondral side of the metaphysis (Fig. 7), separated by the width of the metaphysis from the calcified trabeculæ of the shaft. Since the separation of this line of calcium salts from the other calcified tissues of the bone is complete, and the line itself is so definite, it has been possible to utilize the formation of this deposit as a test to determine the presence of calcium-depositing substances in various natural and synthetic foodstuffs, and a report of the results of such a series of investigations will appear shortly.

organic factor, or of the latter and phosphate. As we have said above, we cannot now be certain whether this organic factor is identical with the anti-xerophthalmic fat-soluble A. It is certain that an amount of butter fat which is more

FIG. 9.



to Fig. 8

Low-power photograph to show a section through the **distal end of a rachitic femur. Rat** on diet of lot 3041 which is low in calcium and in the **organic substance but has,** ***cæteris paribus***, an approximately optimal amount of **phosphate.** ***Ep*****, epiphysis;** ***cart.*****, epiphyseal cartilage;** ***met.*****, metaphysis;** ***dia.*****, shaft.**

It would seem to us that something more than a deficiency of the fat-soluble A is necessary to produce a picture in rats identical with that seen in the bones of rachitic human beings. Bones which most nearly correspond to the rachitic bones of children are those in which osteogenesis is carried on in the face of a deficiency both of calcium and the than adequate to protect an animal from keratomalacia will not affect the calcification of the provisional cartilage, which follows the administration of a very small amount of cod-liver oil. An inadequate supply or the absence of calcium from the diet does not cause the appearance of a pathological picture which corresponds exactly to that of rickets, a fact which

That the animals receiving cod liver oil were in a state of nutritional instability notwithstanding their good external appearance and fertility, is shown by the tendency of the females to collapse and die while nursing a second or third litter of young. Their skeletons were very poorly calcified and when the animals were boiled in water it was impossible to separate even the bones of the pelvis. The femur remained intact but was frequently deformed in second or third generation animals. The skull bones disintegrated in this treatment.

Succeeding generations tend to fail on this diet, but not as rapidly as rats on similar diets containing butter fat even in large amounts (ten to twenty times as much fat as in the cod liver oil diets). The small amount of calcium added in the 5 per cent of milk powder exerted an observable effect in improving the well being of these rats. This is easily seen by comparing Lots 2732 and 2733 (Chart 2) with Lot 2765 (Chart 6).

CHART 7. Lots 2957 and 2958. These groups, and those described in later charts, illustrate the beneficial effects of adding small amounts of calcium to the standard diet employed in most of the experiments here described. A comparison of Chart 1 with Chart 7 shows how marked is the effect in promoting the vitality of rats by the addition of even 0.1 to 0.2 per cent of calcium carbonate to the diet. Lot 2957 failed to grow as well with 0.1 per cent as did Lot 2958 with 0.2 per cent addition. The contrast is much greater in the vigor and capacity to grow of the young produced by these two groups. 0.2 per cent of calcium carbonate made it possible for the second generation to develop fairly well and produce young, whereas the addition of but 0.1 per cent did not enable the young appreciably to grow or to extend their lives beyond about 60 days after weaning. Both groups aged early and had abnormal forms. They were short and stocky. Even 1 per cent of cod liver oil makes rats on this diet develop long, lithe forms, although they fail early.

CHARTS 8 and 9. Lots 2952 (Chart 8) and 2953 (Chart 9) should be compared with Chart 7. The diets were the same except that in Chart 7 the fat addition consisted of 8 per cent of butter fat, whereas in Charts 8 and 9 it was 2 per cent of cod liver oil. Lot 2952 had 0.1 and Lot 2953 had 0.2 per cent of calcium carbonate added. The great superiority of cod liver oil over five to ten times as much butter fat is easily seen.

The general appearance of these animals was superior to the animals fed butter fat. Fertility was high and the mortality was low, although the nursing period was frequently prolonged beyond the normal time. The young did not look sleek and well nourished while depending on the mother, but later when placed on the family diet greatly improved in appearance.

CHARTS 10 and 11. Lot 2959 (Chart 10) should be contrasted with Lot 2954 (Chart 11). These show the growth and fertility of rats fed the basal diet discussed in preceding charts to which 0.3 per cent of calcium carbonate was added. In the former, 8 per cent of butter fat was added, whereas 2 per cent of cod liver oil was added to the latter. Both groups grew well,

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1921, xix, p. 43–47.

21 (1768)

II. The prevention of the development of rickets in rats by sunlight.

By P. G. SHIPLEY, E. A. PARK, G. F. POWERS, E. V. McCOLLUM
and NINA SIMMONDS.

[*From the Department of Pediatrics, Johns Hopkins University,
Baltimore, Md., Dept. of Pediatrics, Yale University, New
Haven, Conn. and School of Hygiene, Johns
Hopkins University, Baltimore, Md.*]

In June, 1919, Huldschinsky[1] reported that the ultraviolet ray exerted a curative action in rickets. The criterion on which he relied was the evidences furnished by the X-ray of calcium deposition at the ends of the long bones. He found that there were definite signs of calcium deposition after four weeks of treatment and that at the end of eight weeks healing was almost complete. In May, 1920, Huldschinsky[2] again reported the curative effects of treatment with the ultraviolet ray in rickets in a series of thirty children, aged between one and one half and six and one half years, who exhibited all clinical manifestations of the disease. In all, healing was accomplished after twenty-two to twenty-six treatments covering a period of two months. In April, 1920, Putzig[3] corroborated the findings of Huldschinsky. He obtained cures by means of the quartz lamp in premature infants suffering from rickets. In July, 1920, Riedel[4] further confirmed Huldschinsky's findings in a series of one hundred children suffering from rickets. In June, 1921, Hess[5] confirmed Huldschinsky's findings in a series of six cases.

The favorable influence of sunlight in rickets has been recognized by some students of the disease for a long time, notably by

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[1] Huldschinsky, K., *Deutsch. Wchnschr.*, 1919, xlv, 712.

[2] Huldschinsky, K., *Ztschr. f. orthop. Chir.*, 1920, lxxxix, 426.

[3] Putzig, H., *TherapHo. lbmonatschr.*, 1920, viii, 234.

[4] Riedel, G., München. med. Wchnschr., 1920, lxvii, 838.

[5] Hess, A. E., and Unger, L. J., *Am. J. Dis. Child.*, 1921, xxii, 186.

Palm[3] (1890), and experimental evidence of its beneficial effect on mineral metabolism in the puppy has been furnished by Raczynsky[4] in 1912. Huldschinsky made use of sunlight together with the ultraviolet ray in two cases of his series and Riedel relied on treatment with sunlight in some of his cases, supplementing with the quartz lamp ray only on sunless days. Hess[5] was the first, so far as we are aware, to demonstrate, by means of the radiograph, that sunlight alone exerts the same curative action as the ultraviolet ray. All the investigations which have been made up to the present time in regard to the curative effects of both the ultraviolet ray and sunlight in rickets have been made on human subjects of the disease and all the evidence has been furnished by means of the radiograph. In order to satisfy ourselves concerning the action of light in rickets as well as actually to see the changes produced in the bones we performed the following experiments.

Eighteen rats about six weeks old and weighing between forty and fifty grams were placed on diet 3,143 which, as previous experience[6] has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings. The ration has the following composition:

Wheat	33.0%
Maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

It contains nearly twice the optimal content of calcium, and is decidedly below the optimum in its content of phosphorus and in fat soluble A. Otherwise, it is well constituted.

Twelve rats placed upon this diet were sent to New Haven, there to be exposed to sunlight. The remaining six rats were retained in Baltimore to be kept as control animals under ordinary laboratory conditions in a large, well-ventilated room completely screened with windows of ordinary glass. The animals treated with the sunlight were divided into two groups and placed in fairly large wire mesh cages. Each clear day the cages were carried out of doors and placed in the sunlight. At first, the weather being warm, the rats were exposed to the sunlight for two short periods of twenty minutes each. Soon, however, the periods were lengthened to six or even more hours. During the experimental period, which covered between sixty-two and sixty-seven days, the rats were exposed to the sunlight on every day except nine. The total exposure to sunlight during the experimental period varied between two hundred and forty-two and two hundred and seventy-three hours. The average daily exposure was four hours.

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[3] Palm, T. A., *The Practitioner*, 1890, xlv, 270–279 and 321–342.

[4] Raczynski, J., *Compt. rend. de L'Association Internationale De Pédiatrie*, Paris, 1913, p. 308.

[5] Hess, A. F., and Unger, L. J., *J. A. M. A.*, 1921, lxxvii, 39.

[6] McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A. *J. Biol. Chem.*, 1921, xlvii, 507.

When first exposed to sunlight, the albinos developed conjunctivitis; the ears of all, in particular the albinos, began to peel; the skin of the tails became sunburned and rough; the hair of some of the albinos acquired a yellowish tint. Long before the experiments were completed it became evident that the animals treated with sunlight were not developing rickets. Though they did not grow normally, they remained extremely active, climbing and darting about the cages. Toward the end of the experiments the males became sexually active; one of the females became pregnant.

The control rats, killed at the expiration of two months, showed all the gross and microscopic evidences of rickets, the characteristic deformities of the thorax, enlargement and distortion of the costochondral junctions, fractures of the shafts, enlargements at the wrists, ankles and knees, and the ends of all the long bones. The bones cut with diminished resistance. On section a deep rachitic metaphysis entirely free from calcium was exposed. Into it the proliferative cartilage extended in irregular prolongations. The trabeculæ were surrounded with broad zones of osteoid.

The rats exposed to the sunlight, on the other hand, showed none of the evidences of rickets. The thorax was not deformed; the costochondral junctions were normal. There were no fractures of the ribs. The ends of the long bones were not enlarged. The long bones cut with great resistance. On microscopic examination the cartilage was normal. The proliferative zone was completely calcified. The trabeculæ were completely calcified. The condition found was normal except that both microscopically and grossly the bone was more delicate than in the rat of corresponding age reared on satisfactory diets. Though the sunshine completely prevented the development of rickets, it did not entirely compensate for the deficiency of phosphorus in the diet, either as regards the growth and development of the rat as a whole or of the skeleton.

There were some noteworthy findings outside the skeleton. An abundance of fat was present. In the control rats the fat was scant. The thymus was only partially involuted. In the control rats it was completely involuted. The spleen was not enlarged.

DISCUSSION.

Sunlight effectually prevents the development of rickets in the rat. We have already shown,[1] as has also Pappenheimer, that cod-liver oil prevents the development of rickets in the rat. As nearly as we can judge from the radiographs furnished by Huldschinsky and others the mode of healing at the cartilage-shaft junction induced by the ultraviolet ray (sunlight) is exactly analogous to that which occurs after the administration of cod-liver oil, as determined by Howland and Park.[2] The time relations are also similar. Huldschinsky found that the ultraviolet ray produced definite evidences of healing at the end of four weeks, and at the end of two months almost complete healing. Howland and Park found that cod-liver oil first gave rise to evidences of healing at the junctions of the cartilage and shaft of the long bones three weeks after the administration was begun and that at the end of about two months the calcification of the diseased ends of the shafts seemed to be complete. Moreover, as the result of the gross and histological examinations made on the rats fed the rickets-producing diet 3,143 but exposed to sunlight it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod-liver oil. Cod-liver oil contains something which is essential for optimal cellular function. Light also contains something which is essential for optimal cellular function. Cod-liver oil or light when made available to an organism previously deprived of either permits the organism to put into successful operation adaptations or defense mechanisms which otherwise would have been ineffectual. Neither cod-liver oil nor light meets the defects in the composition of the diet directly by supplying to the body either calcium or phosphorus but meets them indirectly by so raising the potential of cellular activity as to secure the most efficient utilization possible of those substances available in the body which are directly or indirectly concerned with ossification and calcification.

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[1] Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, Nina. PROC. SOC. EXPER. BIOL. AND MED., xviii, 227, 1921.

[2] Howland, J., and Park, E. A., *Arch. Pediat.*, 1920, xvxvii, 411.

Howland, J., and Park, E. A., *Bull. Johns Hopkins Hosp.*, November, 1921. (To be published.)

low intake of this element there is a correspondingly high requirement of the substance which we have spoken of as the calcium-depositing factor, but which is more than this. It exerts a distinct influence on the anatomic elements of the growing bone and enables the osteoblasts to form approximately the optimal amount of osteoid tissue. It leads to the deposition of calcium phosphate in a degree which is not possible in its absence when the calcium content of the diet is very low. It improves the general well being of the animals.

The most important point brought out by the records in this chart is that when 0.5 per cent of calcium carbonate was added to the diet, which still contained only about half the optimal content of calcium, even so small an amount of butter fat as 3 per cent sufficed to enable the animals to grow in a normal manner and to exhibit normal vitality as shown in fertility and success in rearing young. The second generation was somewhat undersized but was fertile.

This chart should be compared with Chart 1. Lot 2934 had nearly seven times the content of butter fat which was consumed by Lot 2839, yet in the absence of a calcium supply exceeding that furnished by a cereal and legume seed mixture they were not protected by this high butter fat intake.

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2. Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, N., Studies on experimental rickets. VII. The relative effectiveness of cod liver oil as contrasted with butter fat for protecting the body against an insufficient calcium supply in the presence of a normal phosphorus supply, *Am. J. Hyg.*, 1921, i, 512.
3. Hart, E. B., Steenbock, H., and Hoppert, C. A., Dietary factors influencing calcium assimilation. I. The comparative influence of green and dried plant tissues, cabbage, orange juice, and cod liver oil on calcium assimilation, *J. Biol. Chem.*, 1921, xlviii, 33.
4. Park, E. A., Powers, G. F., Shipley, P. G., McCollum, E. V., and Simmonds, N., Studies on experimental rickets. XIV. The prevention of the development of rickets by sunlight, *J. Am. Med. Assn.*, 1922, lxxviii.

EXPLANATION OF PLATE 1.

FIG. 1 shows the appearance of a rat which was restricted to Diet 2821 (Chart 3). This diet was faulty in that its calcium content was too low and the phosphorus near the optimal. The fat in the diet consisted of 5 per cent of butter fat. Note the poor coat and general appearance of inferior development. This picture should be contrasted with Fig. 2, which had the same diet but with 2 per cent of cod liver oil and 3 per cent of butter fat.

FIG. 2. This rat from Lot 2822 was the same age as that shown in Fig. 1, and was confined to its experimenal diet for the same number of days (155). Its diet differed from that shown in Fig. 1 only in that 2 per cent of cod liver oil replaced 2 per cent of butter fat. Note the superior development, the good coat, and general appearance of physical well being brought about by the small amount of cod liver oil.

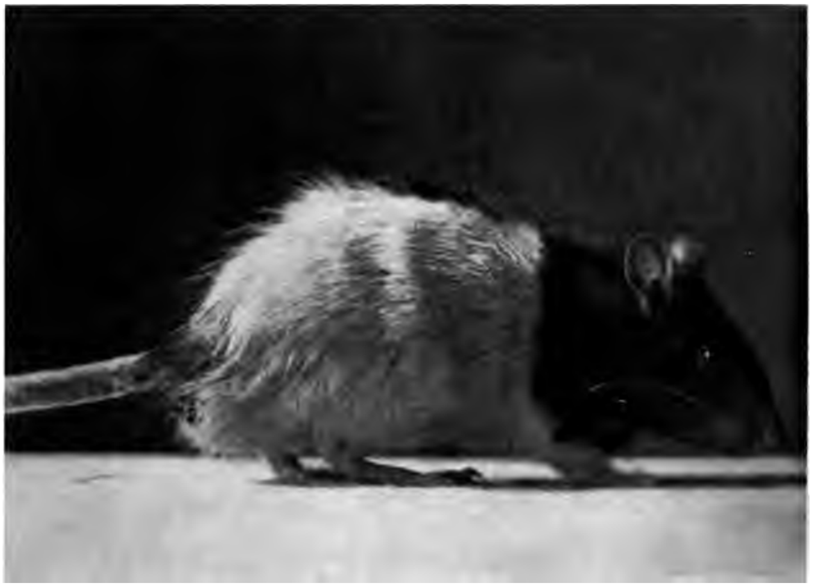

Fig. 1.

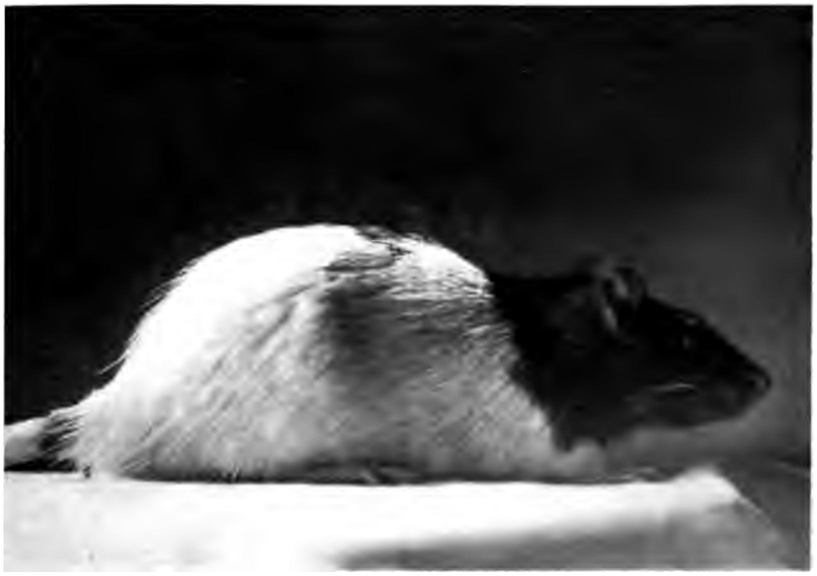

Fig. 2.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. XII.)

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The

The Function of the Organic Factor as Exemplified by Cod Liver Oil XIII

P. G. SHIPLEY, E. A. PARK,
E. V. McCOLLUM AND NINA SIMMONDS
BALTIMORE

Cod liver oil, we have repeatedly observed with different diets, protects growing rats against the injurious effects of lack of calcium and enables them to grow and appear well nourished for a considerable period, where they would fail to grow and would be very inferior with even much greater amounts of butter fat instead of the cod liver oil.

It is especially remarkable that whereas 3 per cent of butter fat suffices when added to food mixtures of the type under discussion to meet all the requirements of the rat for fat-soluble A and for the specific calcium-depositing factor, if there be one, when the diet contains favorable concentrations of calcium, 10 to 20 per cent of butter fat fails to shield them in a manner at all comparable with 1 per cent of cod liver oil. Again, it is remarkable that animals specifically fasted for calcium are protected as effectively by 1 per cent of cod liver oil as by 3 per cent or higher planes of intake.

We do not believe that our samples of butter fat could differ in the concentration of the calcium-depositing substance, since, as will be shown in later charts, when the diet contains calcium in amounts ranging from 0.1 to 0.5 per cent of the carbonate, butter fat becomes adequate to supply the nutritive needs of the body and the differences in the effects of cod liver oil and of butter fat in the diet tend to disappear.

CHART 3. Lot 2821 was fed a diet essentially comparable to those discussed in the preceding charts except that its content of 20 per cent of casein raised the phosphorus content of the food mixture. Like the preceding diets it was very poor in calcium. 5 per cent of butter fat did not suffice to protect the animals against lack of calcium. This chart should be compared with Chart 4, Lot 2822, which differed significantly only in containing 2 per cent of cod liver oil and 3 per cent of butter fat.

Lot 2821 was very poorly nourished and became badly deformed. The coats of these rats were rough and thin (see Fig. 1) and they aged very early.

CHART 4. Lot 2822 had a diet exactly similar to that of Lot 2821 (Chart 3) except that 2 per cent of cod liver oil replaced 2 per cent of butter fat. This modification of the diet made a remarkable difference in their growth and well being (see Fig. 2). The former were stunted, infertile, and short lived. The latter grew to full normal size, presented a well nourished appearance, and were fairly fertile, and succeeded in rearing a considerable number of their young to the weaning age. The young were puny, potbellied, almost completely stunted in growth, and died early. The mothers declined rapidly after nursing two or three litters. The males, while well nourished for an interval following the completion of growth, soon presented a poorly nourished appearance and aged early.

The effect of the cod liver oil was to make the animals in some degree immune for a time to the injurious effects of lack of calcium. Even ten times as much butter fat could not do this. If a small addition of calcium were made to this diet the butter fat would supply sufficient of some organic which without it would have been ineffectual. It does not meet the defects in the inorganic composition of the diet directly by supplying to the body either calcium or phosphorus,[1] but meets them indirectly by so raising the potential of the cell as to secure the most efficient utilization possible of those substances available in the body which are directly or indirectly concerned with ossification and calcification. It seems to bring about normal ossification and calcification or else the closest approximation to normal ossification and calcification compatible with the marked variations from the optimal in the amounts of certain of the inorganic constituents of the diet. It does not regulate the inorganic metabolism of the body but permits the body's own regulatory mechanisms to operate with increased efficiency and economy.

The mechanism by which cod liver oil exerts this influence on the processes of ossification and calcification we shall not attempt to discuss.

If we are correct in the interpretation of our experiments bearing on this point—which we might add have thus far been concerned chiefly with variations in the calcium-phosphorus ratio in the diet, and are not sufficiently comprehensive to permit the formulation of absolute conclusions—we believe that the theory regarding the function of the active principle contained in cod liver oil and in other substances is of considerable significance. In the first place, the theory, if correct, indicates that a substance possessing properties comparable to those ordinarily ascribed to the secretions of the ductless glands and as essential as any of them to normal growth and development exists outside the body and must be obtained by the body through the food. In the second place, the theory opens the door to a better understanding of the nature of rickets and allied diseases or affections of the skeleton. When the diet is deficient in the organic substance, defects in its salt composition are permitted to extend their injurious influence to the cartilage and the bone. In other words, when faults in the salt composition of the diet of a nature favorable to the development of rickets, osteomalacia or anosteogenic osteoporosis happen to be present, the deficiency in the diet of the substance permits them to develop. Viewed in this light rickets is a dietary disease due to a faulty composition, probably of a specific nature of its mineral elements and an insufficiency of the organic substance.

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[1] In cod liver oil there is a trace of phosphorus.

We believe that the theory in regard to the function of the organic factor, as exemplified by cod liver oil, explains the conflicting results of experiments performed by Mellanby, Sherman and Pappenheimer, Hess, and ourselves. It makes it possible to understand how the withdrawal of the organic factor from the diet may or may not be followed by the development of rickets according as the composition of the mineral portion of the diet is favorable or unfavorable for that development. It also makes it possible to understand how cod liver oil unaided when added to the diet may prevent the development of rickets or heal a rickets already established.

DISCUSSION

Dr. Hess: Dr. Park speaks of the organic factor. Evidently he does not want to label it as identical with the fat-soluble vitamin. I do not believe that the fat-soluble vitamin can be considered the antirachitic factor as stated by Mellanby and the British Research Committee. I have taken this position because it is evident that infants receiving large amounts of fat-soluble vitamin do develop rickets. Furthermore, it is very questionable whether rickets is to be considered as purely a dietetic deficiency disease. There may be a potential deficiency. In other words the diet may contain sufficient chemical substances but they may be so interrelated that they do not benefit the body. I should like to ask Dr. Park whether he draws a distinction between the fat-soluble vitamin and the organic factor or whether he thinks the fat-soluble vitamin is the organic factor. My experience with infants during the last year has confirmed the opinion expressed a year ago. The experiments on rats of McCann, Pappenheimer and myself indicate that there is an organic factor that has to do with the osteogenesis of bone. In an absence of the fat-soluble vitamin rickets did not develop but there was a lack of osteogenesis. Rats were fed a diet adequate in every way except that it did not include the fat soluble vitamin and they developed an irregular osteogenesis but not rickets. When fat-soluble vitamin was added there was growth and cure of the eye condition (xerophthalmia). I am in agreement with Dr. Park that an organic factor plays a role but it is not the fat-soluble vitamin.

I should like to ask Dr. Park whether he believes that animals or infants can do without this organic factor or whether he considers it as merely regulatory; whether if the diet is complete in other respects infants can do without any of this organic matter.

Dr. Chapin: I am quite unable to discuss this paper from the etiologic chemical standpoint but I should like to speak from the clinical standpoint. I think there may be a deficiency in the food but it is the bad hygiene that supplements it. That has been called to my attention on the lower East Side where almost all Italians have rickets. They have come in and taken the rear tenements. First the Irish came in years ago and took these rear tenements, but now they have become our policemen and aldermen and are our rulers, and the Italians have taken these old tenements. Now the Italian babies nearly all have rickets. It is true they have dietetic errors but that is only part of it. Rickets is very rare in Italy where the climate is mild and there is plenty of light and sunshine, though they have the same errors of diet there as here. That leads me to believe that while the diet is partly responsible, there is something else which is not accounted for by the dietetic deficiency.

substance intimately associated with the development of the osseous system, and the animals would develop normally. In order to differentiate between the nutritive value of cod liver oil and of butter fat *in a qualitative way* it must be administered with a diet poor in calcium, for with an adequate supply of calcium and phosphorus in the food either serves equally well to supplement such diets as are here discussed.

CHART 5. Lot 2766. This and succeeding groups of experimental animals illustrate the comparative value of cod liver oil and of butter fat in the presence of suboptimal amounts of calcium, where the phosphorus content of the diet is near the optimum. A small amount of calcium (0.045 gm.) was added in the 5 per cent of milk powder but this was too small to contribute much to the well being of the animals.

These records should be compared with those of Chart 6 (Lot 2765), whose diet was almost identical except that it contained 1 per cent of cod liver oil instead of 10 per cent of butter fat. The rats were protected in a remarkable way against the effects of lack of calcium by this small amount of cod liver oil. 10 per cent of butter fat with this diet afforded some protection, but although this amount is at least three times that required to meet all the needs of the rat for protection against ophthalmia and to enable it to develop a normal skeleton when the diet contains the optimal amount of calcium, it fails to supply enough of some substance intimately concerned with bone formation when the calcium intake is low. These results point to the existence of a specific calcium-depositing substance distinct from fat-soluble A (antixerophthalmic substance).

On this diet the animals grew slowly but never attained the full adult size. The females were capable of producing several litters each but the infant mortality was high and they early developed signs of senility. The second generation confined to this food supply was greatly stunted and inferior. Their forms were very short and stocky. They had large deposits of body fat.

CHART 6. Lot 2765 should be contrasted with Lot 2766 (Chart 5). The significant difference in the composition of the diets of these two groups was in the nature of the fats which they contained. Both diets were far below the optimal in their content of calcium, but were otherwise well constituted. 1 per cent of cod liver oil very effectively protected these animals against the harmful effects of calcium starvation because of its content of some organic substance which appears to be distinct from that substance (fat-soluble A) which is essential for growth and is a specific agent in preventing ophthalmia of dietary origin. The protection afforded by the cod liver oil is not complete. It consists in enabling growth to proceed and causes the animals to appear externally to be well nourished. This is very apparent when we contrast the animals described in Chart 5, which had the same diet with 10 per cent of butter fat, with those in Chart 6, which had 1 per cent of cod liver oil. This contrast we have repeatedly observed in rats fed other diets low in calcium where fat-soluble A was in one case supplied by cod liver oil and in the other by butter fat.

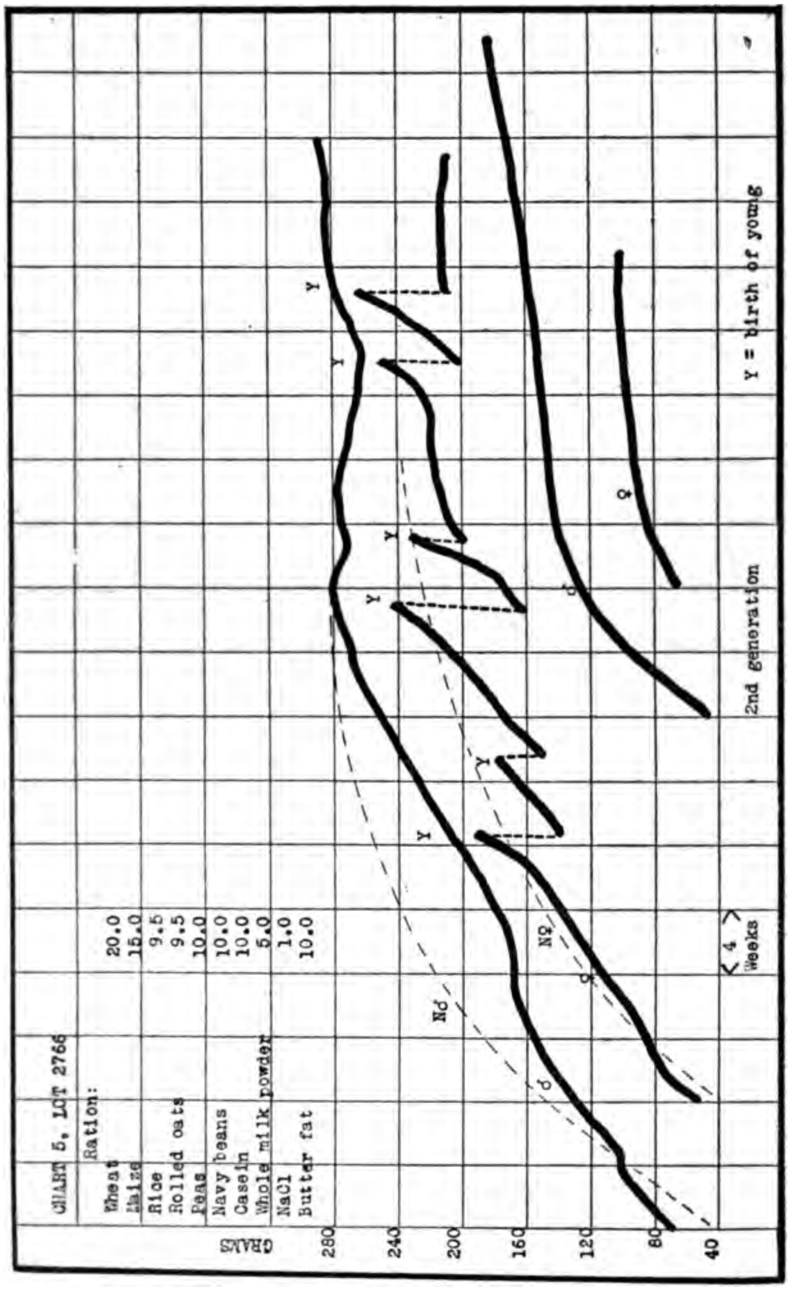
CHART 5, LOT 2766
Ration:
Wheat 20.0
Maize 15.0
Rice 9.5
Rolled oats 9.5
Peas 10.0
Navy beans 10.0
Casein 10.0
Whole milk powder 5.0
NaCl 1.0
Butter fat 10.0
GRAMS
280
240
200
160
120
80
40
< 4 > Weeks
2nd generation
Y = birth of young
Y
N♂
N♀
♂
♀

THE PREVENTION OF THE DEVELOPMENT OF RICKETS IN RATS BY SUNLIGHT

STUDIES ON EXPERIMENTAL RICKETS: XIV *

G. F. POWERS, M.D., AND E. A. PARK, M.D.,
Assistant Professor and Professor in Pediatrics, Respectively, Yale University School of Medicine
NEW HAVEN, CONN.

P. G. SHIPLEY, M.D.
Instructor in Pediatrics, Johns Hopkins University Medical Department
AND
E. V. McCOLLUM, PH.D., AND NINA SIMMONDS, S.B.
Professor of Biochemistry and Associate in Biochemistry, Respectively, Department of Chemical Hygiene, Johns Hopkins University School of Hygiene and Public Health
BALTIMORE

HISTORICAL: LIGHT IN THE CURE OF RICKETS AND TETANY

In 1904, Buchholz[1] published a paper on the treatment by means of light of rickets and certain other diseases occurring in children. He used the "Glühlicht" (Kellogg) which, he stated, was poor in chemically active rays. The heat rays were in part absorbed by a special kind of glass filter interposed between lamp and patient. He considered that the action of the lamp depended in part on the heat rays, in part on the light rays. Whatever the exact composition of the light may have been, Buchholz reported favorable results from its use in rickets. Altogether he treated with it sixteen rachitic children. They all showed general improvement. They ate and slept better; they became more active and began to walk. Sweating of the head decreased, and their backs became straight. The work of Buchholz seems to have commanded little, if any, attention.

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* Read before the American Child Hygiene Association at the Twelfth Annual Meeting at New Haven, Conn., Nov. 2-5, 1921.

[1] Buchholz, E.: Ueber Lichtbehandlung der Rachitis und anderer Kinderkrankheiten, Verhandlungen der Gesellschaft für Kinderheilkunde in der Abteilung für Kinderheilkunde der 76 Versammlung der Gesellschaft Deutscher Naturforscher und Aerzte in Breslau, **21**: 116, 1904.

The fact that the development of the healing process in the bones of rachitic children could be traced by means of the roentgen ray has been understood in Germany for a number of years. In 1910, in an atlas devoted to rickets Fraenkel and Lorey [2] published roentgenograms of the bones of rachitic children to illustrate all stages of healing and of healing and relapse. Under these circumstances it is remarkable that the roentgen ray was not earlier employed to detect the therapeutic effects of drugs and other forms of treatment in diseases affecting the skeleton. Phemister,[3] in this country, applied the method to the study of the effects of phosphorus on growth and ossification in health and disease (1918), and to its use Huldschinsky owed the success of his efforts in demonstrating that the light emitted by the mercury vapor quartz lamp exerts a curative action in rickets.

In a preliminary communication, which appeared in June, 1919, Huldschinsky [4] reported that the ultraviolet ray exerted a curative action in rickets. His material consisted of four children, aged between 2¼ and 4¼ years, who had advanced rickets. Three rachitic children, untreated with the quartz lamp, served as control subjects. After four weeks of treatment, calcium deposition at the ends of the long bones of the extremities could be demonstrated in roentgenograms, and at the end of two months the healing process seemed to be almost complete. Huldschinsky complicated the interpretation of the results of the experiments by the daily administration of 1 gm. of calcium phosphate. Since, however, the rachitic children who were not irradiated failed to show any evidence of calcium deposition in the skeleton as the result of the calcium phosphate administration, Huldschinsky concluded that the beneficial effects were to be attributed entirely to the ultraviolet ray.

In December, 1918, Winkler [5] reported the favorable effects of treatment of rickets with the roentgen ray. He used a medium soft tube at a focal distance of about

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[2] Fraenkel, E., and Lorey, A.: Archiv und Atlas der normalen und pathologischen Anatomie in typischen Röntgenbild, Hamburg, Lucas Gräfe and Sillem, 1910.

[3] Phemister, D. B.: The Effect of Phosphorus on Growing, Normal and Diseased Bones, J. A. M. A. **70:** 1737 (June 8) 1918.

[4] Huldschinsky, K.: Heilung von Rachitis durch künstliche Höhensonne, Deutsch. med. Wchnschr. **45:** 712, 1919.

[5] Winkler, F.: Ueber die Strahlungstherapie der Rachitis, Monatschr. f. Kinderh. **15:** 520 (Dec.) 1918.

20 cm. The exposure did not exceed ninety seconds, and was repeated every other day. The treatment was at first directed against the craniotabetic lesions of the head. After five or six treatments, Winkler observed that the sweating of the head came to an end and sleep was improved. As the treatment progressed, laryngospasm and the "tendency to convulsions" disappeared. The craniotabes vanished. The teeth erupted. Bulgings of the costochondral junctions disappeared. Calcium deposition occurred at the ends of the radius and ulna, as was plainly evident in the roentgenograms. Winkler's work seems to have been done without knowledge of the work of Huldschinsky.

In April, 1920, Putzig[6] corroborated the findings of Huldschinsky. He does not state the number of rachitic children treated by means of the quartz lamp, but observes that he effected cures through its use in premature infants suffering from rickets. In the same month, Huldschinsky's findings received further confirmation from Karger.[7]

In May, 1920, Huldschinsky[8] reported the results of more extensive investigations of the effects of the ultraviolet ray in rickets. His material had now been increased to thirty children. He administered calcium phosphate or calcium chlorid to twenty of the children, but to ten did not give calcium in any form. In some of the thirty children treated the rickets was recent, in others of long standing; the ages of the children varied between 1½ and 6½ years. In all, healing was accomplished in from twenty-two to twenty-six treatments covering a period of two months. As the result of his further investigations, Huldschinsky concluded that there could be no longer any doubt that the ultraviolet ray exerted a specific therapeutic influence in rickets.

In July, 1920, Riedel[9] further confirmed Huldschinsky's findings in a series of 100 rachitic children.

In September, 1920, Sachs[10] reported that treatment with the ultraviolet ray cured latent tetany. His evi-

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6. Putzig, H.: Die Behandlung der Rachitis mit künstlicher Höhensonne, Therap. Halbmonatschr. **8**: 234 (April) 1920.

7. Karger, P.: Zur Kenntniss der zerebralen Rachitis, Monatschr. f. Kinderh. **18**: 21, 1920.

8. Huldschinsky, K.: Die Behandlung der Rachitis durch Ultraviolettbestrahlung, Ztschr. f. Orthop. Chir. **89**: 426 (May) 1920.

9. Riedel, G.: Die Erfolge der Quartzlichtbestrahlung bei Rachitis, München. med. Wchnschr. **67**: 838 (July) 1920.

10. Sachs, F.: Untersuchungen über den Einfluss des Ultraviolettlichtes auf die latente Saüglingstetanie, Jahrb. f. Kinderh. **93**: 167 (Sept.) 1920.

dence consisted in the disappearance under treatment of the mechanical and electrical excitability of the peripheral nerves. His conclusions were based on the result of treatment in seven cases. No medication was given.

In the same month Huldschinsky[11] reported cures of manifest tetany by means of irradiation with the quartz lamp. No medication was given. After the first application of the rays, the manifest symptoms disappeared. In no case did the laryngeal stridor or the convulsions recur. The latent symptoms, i, e., the Chvostek and the characteristic electrical reactions, disappeared within four days in one case, but lingered in

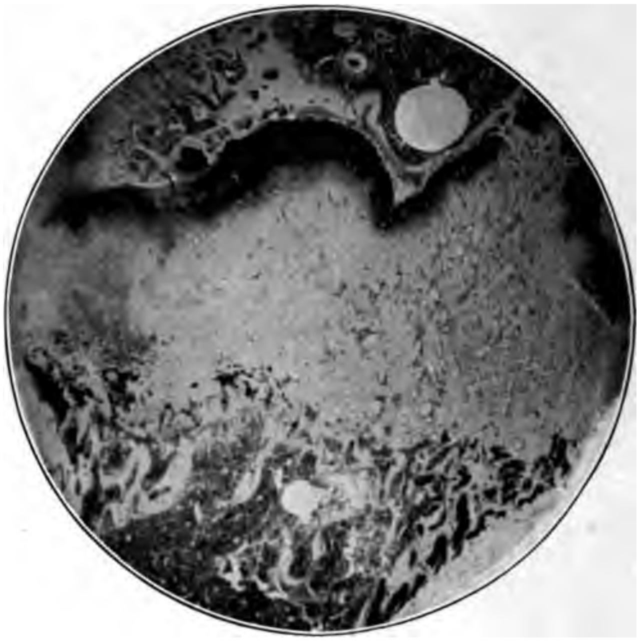

Fig. 1.—Photomicrograph of a rachitic bone from a control rat which was fed Diet 3143 and not exposed to sunlight. The epiphyseal cartilage is uncalcified and a broad intermediate zone or metaphysis has formed between it and the shaft. There is a pathologic overproduction of osteoid tissue in the shaft.

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[11] Huldschinsky, K.: Die Beeinflussung der Tetanie durch Ultraviolettlicht, Ztschr. f. Kinderh. **26**: 5 (Sept.) 1920.

the other cases for from two to four weeks. By this time the number of rachitic children cured by Huldschinsky through the use of the quartz lamp had risen to 105.

In August, 1921, Sachs [12] reported the cure of eight children having severe tetany, by means of the ultraviolet ray.

In May, 1921, Erlacher [13] reported the cure of rickets in forty-two cases by means of treatment with the ultraviolet ray. The subjects varied in age from 1 to 7 years, and presented all the symptoms and were in all stages of the disease. The number of treatments necessary to effect a cure varied between forty and sixty. Marked general improvement was noted coincidently with the healing of the rickets.

In June, 1921, Mengert [14] announced the successful use of the quartz lamp as a prophylaxis against rickets. His work, which is not impressive, consisted in the treatment with the quartz lamp of eighteen infants, aged between 1 and 2 months. One infant was 2½ months old. Ten were premature. Some died from intercurrent disease before the prophylactic treatment was complete. No one of the children treated developed rickets as determined by the roentgen ray.

In January, 1921, Hess and Unger [15] reported the cure of rickets in six children by means of the ultraviolet ray. They conceived of the possibility of the curative action of the ultraviolet ray in rickets in 1917 and actually treated with it at that time six children. Unfortunately, however, they failed to discover that the tenement was valuable. They did not use roentgenographic evidence of healing at the ends of the long bones of the extremities, which was objective and measureable. In 1920 they first described these experiments performed in 1917:

> Infants in glass cubicles were not spared more than those in the regular wards. With this question in mind, in the spring of 1917 five children were given daily treatments of

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[12] Sachs, F.: Die Heilung der Säuglingstetanie durch Bestrahlung mit Ultraviolettlicht, München. med. Wchnschr. **68**: 985 (Aug.) 1921.

[13] Erlacher, P.: Ueber Heilerfolge bei Rachitis nach Quarzlichtbestrahlung, Wien. klin. Wchnschr. **34**: 241 (May) 1921.

[14] Mengert, E.: Ueber vorbeugende Höhensonnenbestrahlung gegen Rachitis, Deutsch. med. Wchnschr. **47**: 675 (June) 1921.

[15] Hess, A. F., and Unger, L. J.: The Clinical Rôle of the Fat-Soluble Vitamin: Its Relation to Rickets, J. A. M. A. **74**: 217 (Jan. 24) 1920.

violet ray, the mercury vapor quartz lamp being used for this purpose. Their entire bodies were exposed for twenty minutes, so that they soon became brown, as if well tanned by the sun. This therapy, which was carried out with regularity for three months in infants about 1 year of age, did not lead to a definite improvement in the rickets, nor did it benefit their general condition. Violet ray cannot be con-

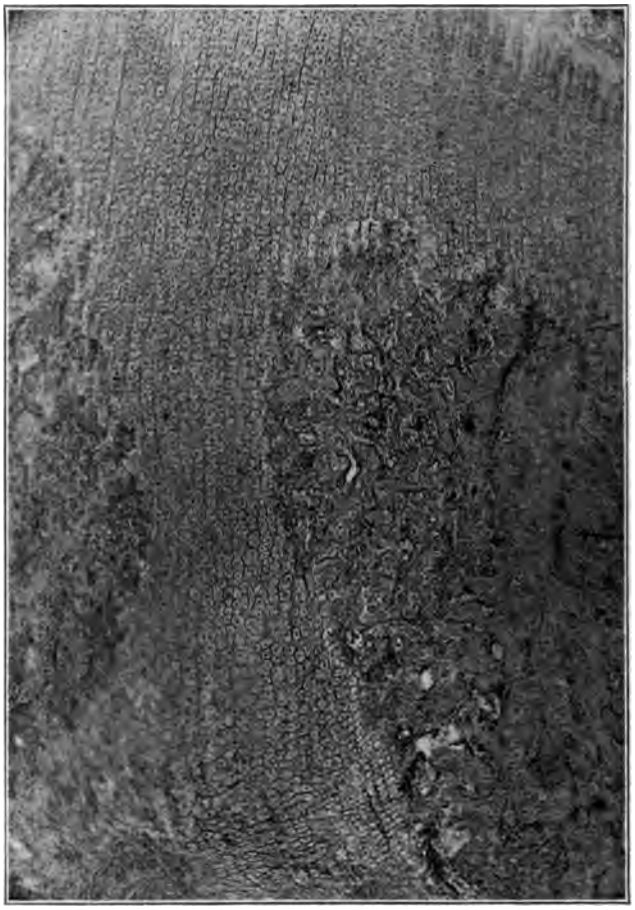

Fig. 2.—Section of a bone from a control animal at the epiphyseo-diaphyseal junction, showing severe rachitic lesions. This animal had been fed for two months on Diet 3143. The picture shows the abnormal persistence of the cartilage and its invasion by blood vessels from the shaft and marrow elements. There is a pathologic formation of osteoid tissue in which cartilage cells (indicated by arrow) are embedded.

sidered the equivalent of heliotherapy. But the fact that rickets is exceptional in the arctic region, where there is a lack of sunlight for the greater part of the year, is a strong argument against its predominant influence.

Moist, foggy climates were regarded by Glisson[16] as an etiologic factor in the production of rickets in children.

The true value of sunlight as a therapeutic agent in rickets was set forth by Palm[17] in 1890 as the result of a noteworthy topographic study of the incidence of the disease. We quote the remarkable recommendations for the eradication of rickets from the community which form the conclusion of his article:

> In conclusion, as practical results of this inquiry, I would urge the following:
>
> 1. The establishment of means for having systematic and exact records of the sunshine in the heart of our great cities as well as at favorite health resorts. A sunshine recorder at an observatory on some hilltop near a large city is no guide to the amount of sunshine that reaches the streets and alleys of smoky cities. It is important that the sunshine recorder be of the form which indicates the chemical activity of the sun's rays rather than its heat.
>
> 2. The removal of rachitic children as early as possible from large towns to a locality where sunshine abounds and the air is dry and bracing.
>
> 3. The establishment of a sanatorium for poor rickety children in some such locality, where the severe development of the disease may be averted, and much life and health saved by timely treatment.
>
> 4. The systematic use of sun-baths as a preventive and therapeutic measure in rickets and other diseases.
>
> 5. That when a mother has once borne a child which has become rachitic, preventive treatment of the disease in her future children should be adopted if possible by change of climate and mode of life in the mother, nothing urged above being inconsistent with the belief that the mother's state of health brought about by the same causes predisposes her offspring to rickets.
>
> 6. The education of the public to the appreciation of sunshine as a means of health. Many persons seem to prefer darkness to light in their dwellings out of ignorance, thoughtlessness, or even an economic regard for carpets and curtains. Let people understand that sunlight in the dwelling not only

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[16] Glisson, F.: De rachitide, London, 1650.

[17] Palm, T. A.: The Geographical Distribution and Aetiology of Rickets, Practitioner, London 45: 271 and 321, 1890.

reveals unsuspected dirt, but is Nature's universal disinfectant, as well as a stimulant and tonic. Such knowledge will also stimulate efforts for the abatement of smoke, and for the multiplication of open spaces, especially as playgrounds for the children of the poor.

Experimental evidence for the favorable action of sunlight on the mineral metabolism was furnished by Raczynski[18] in 1912. On account of its importance, we quote Raczynski's brief communication in its entirety:

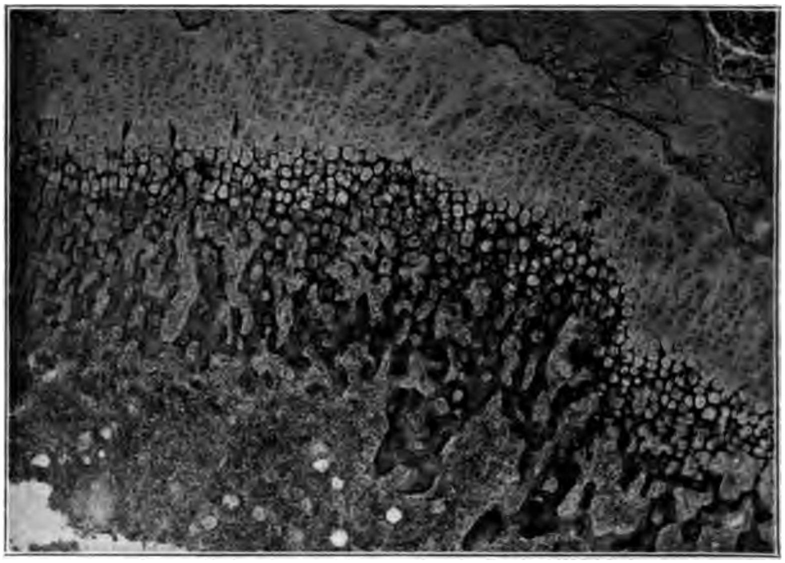

Fig. 3.—Section from a long bone of a rat which had been on the same diet (3143) as the control shown in Figure 1. The cartilage is narrow, regular and well calcified. The marrow is normal, and the bony trabeculae of the shaft, while somewhat fewer than those of a normal bone, are well calcified and show evidences of normal osteoblastic activity. This animal was exposed to sunlight in the manner described in the test.

There are numerous investigations which have had as their object the determination of the etiology of rickets, but there exists none which consists in an experimental inquiry into the action of the sun in that disease.

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[18] Raczynski, J.: Communications sur le rachitisme: I, Recherches experimentales sur le manque d'action du soleil comme cause du rachitisme, Compt. rend. de l'Association internationale de pédiatrie, 1912, p. 308.

That the animals receiving cod liver oil were in a state of nutritional instability notwithstanding their good external appearance and fertility, is shown by the tendency of the females to collapse and die while nursing a second or third litter of young. Their skeletons were very poorly calcified and when the animals were boiled in water it was impossible to separate even the bones of the pelvis. The femur remained intact but was frequently deformed in second or third generation animals. The skull bones disintegrated in this treatment.

Succeeding generations tend to fail on this diet, but not as rapidly as rats on similar diets containing butter fat even in large amounts (ten to twenty times as much fat as in the cod liver oil diets). The small amount of calcium added in the 5 per cent of milk powder exerted an observable effect in improving the well being of these rats. This is easily seen by comparing Lots 2732 and 2733 (Chart 2) with Lot 2765 (Chart 6).

CHART 7. Lots 2957 and 2958. These groups, and those described in later charts, illustrate the beneficial effects of adding small amounts of calcium to the standard diet employed in most of the experiments here described. A comparison of Chart 1 with Chart 7 shows how marked is the effect in promoting the vitality of rats by the addition of even 0.1 to 0.2 per cent of calcium carbonate to the diet. Lot 2957 failed to grow as well with 0.1 per cent as did Lot 2958 with 0.2 per cent addition. The contrast is much greater in the vigor and capacity to grow of the young produced by these two groups. 0.2 per cent of calcium carbonate made it possible for the second generation to develop fairly well and produce young, whereas the addition of but 0.1 per cent did not enable the young appreciably to grow or to extend their lives beyond about 60 days after weaning. Both groups aged early and had abnormal forms. They were short and stocky. Even 1 per cent of cod liver oil makes rats on this diet develop long, lithe forms, although they fail early.

CHARTS 8 and 9. Lots 2952 (Chart 8) and 2953 (Chart 9) should be compared with Chart 7. The diets were the same except that in Chart 7 the fat addition consisted of 8 per cent of butter fat, whereas in Charts 8 and 9 it was 2 per cent of cod liver oil. Lot 2952 had 0.1 and Lot 2953 had 0.2 per cent of calcium carbonate added. The great superiority of cod liver oil over five to ten times as much butter fat is easily seen.

The general appearance of these animals was superior to the animals fed butter fat. Fertility was high and the mortality was low, although the nursing period was frequently prolonged beyond the normal time. The young did not look sleek and well nourished while depending on the mother, but later when placed on the family diet greatly improved in appearance.

CHARTS 10 and 11. Lot 2959 (Chart 10) should be contrasted with Lot 2954 (Chart 11). These show the growth and fertility of rats fed the basal diet discussed in preceding charts to which 0.3 per cent of calcium carbonate was added. In the former, 8 per cent of butter fat was added, whereas 2 per cent of cod liver oil was added to the latter. Both groups grew well,

lay and Watson,[19] on the etiology of rickets, Neve [20] called attention to the fact that rickets was exceedingly rare in Srinagar (India), where infants live under the worst conditions of hygiene and diet, but where sunlight, to which they are almost constantly exposed, abounds. He stated that the only case of rickets which he had seen there was in an English child. A cure took place when the gloomy house in which the patient lived was exchanged for one which was well lighted by the sun.

The favorable effect of sunlight in rickets as determined by clinical observation has been recently emphasized by Feer.[21] He called attention to the marked benefit which accrued in rickets from exposure to the sun's rays in the Swiss Alps. He made reference to the excellent results which had been obtained in the treatment of rickets with sunlight during the past ten years at the Zurich Sanatorium, and also to the investigation of Neumann [22] in 1909 in regard to the incidence of rickets and tetany in the Swiss Alps. Neumann had pointed out the great rarity of both rickets and tetany in Arosa, 1,740 meters (5,709 feet) above the sea level, and its frequency at Davos, at an altitude of 1,560 meters (4,285 feet) and only four and one-half hours distant. At Arosa the children lived for a large part of the time outdoors; but in Davos they lived largely indoors under the crowded conditions which are found generally in cities.

Huldschinsky made use of sunlight together with the ultraviolet ray in two cases of his series, and Riedel relied on treatment with sunlight in some of his cases, supplementing with the quartz lamp only on sunless days.

Hess and Unger [23] were the first, so far as we are aware, to demonstrate by means of the roentgen ray that sunlight alone possesses the same curative action as the light of the quartz lamp in the rickets of human beings. They state that they exposed five infants with rickets to the direct action of sunlight for periods vary-

ing from one-half hour to several hours daily, whenever the sunlight was available. Different parts of the body were in turn subjected to the action of the sun's rays. In one of the cases the patient was exposed to the sunlight only on seven occasions for a total period of twenty-five hours. The general condition of the infants, as well as the diseased condition in the bones,

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[19] Paton, D. N.; Findlay, L., and Watson, A.: Observations on the Cause of Rickets, Brit. M. J. **2**: 625 (Dec. 7) 1918.

[20] Neve, E. F.: The Etiology of Rickets, Brit. M. J **1**: 518, 1919.

[21] Feer, E.: Die Einwirkung des Höhenklimas auf das kranke Kind, Schweiz. med. Wchnschr. **51**: 438, 1921.

[22] Neumann, H.: Der Säugling im Hochgebirge, Deutsch. med. Wchnschr. **35**: 2167, 1909.

[23] Hess, A. F., and Unger, L. J.: The Cure of Infantile Rickets by Sunlight, J. A. M. A. **77**: 39 (July 2) 1921.

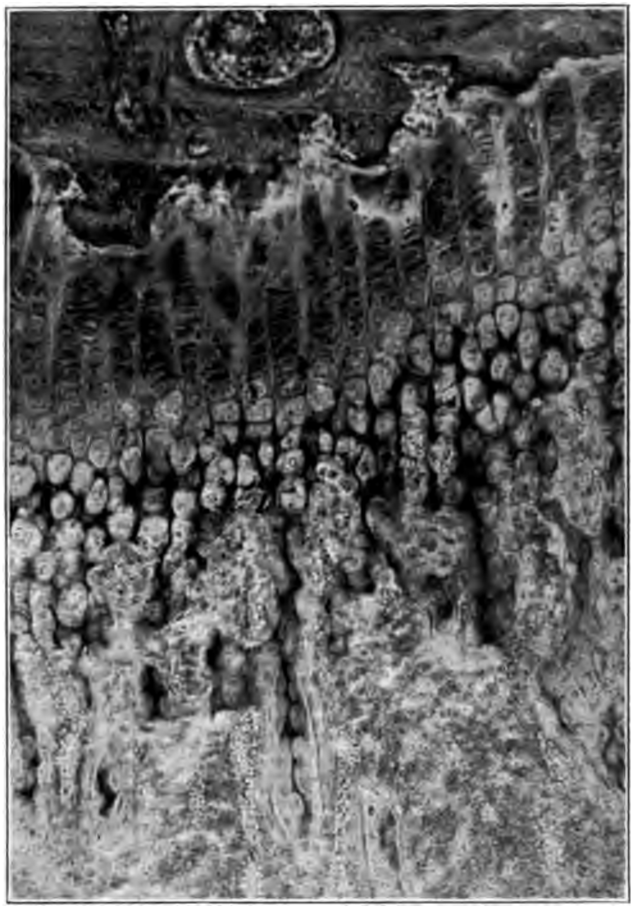

Fig. 4.—Epiphyseodiaphyseal junction of a bone from a rat on a rickets-producing diet, but exposed to sunlight. Note the complete calcification of cartilage and trabeculae.

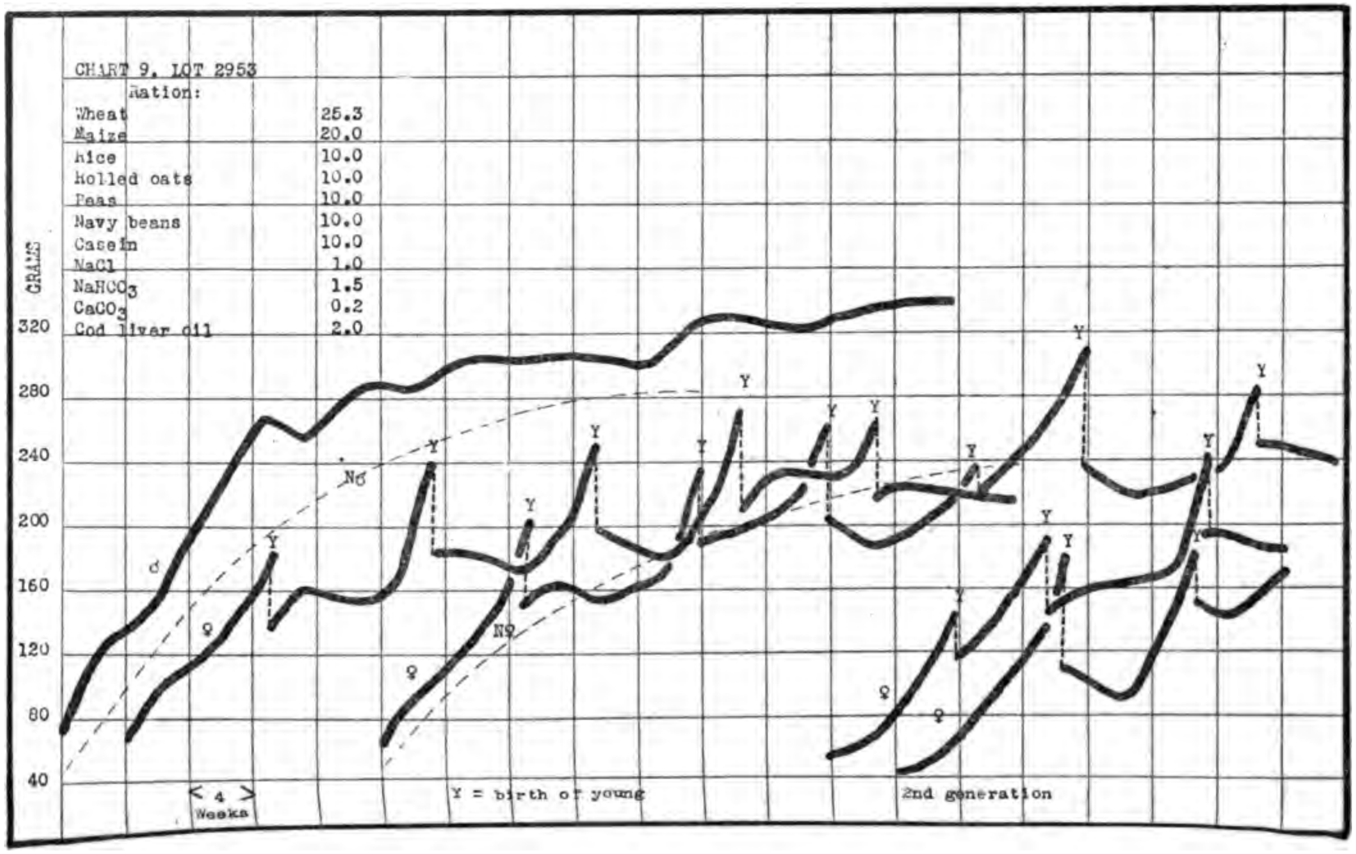
CHART 9. LOT 2953
Ration:
Wheat 25.3
Maize 20.0
Rice 10.0
Rolled oats 10.0
Peas 10.0
Navy beans 10.0
Casein 10.0
NaCl 1.0
NaHCO3 1.5
CaCO3 0.2
Cod liver oil 2.0
GRAMS
320
280
240
200
160
120
80
40
♂
♀
N♂
N♀
Y
< 4 >
Weeks
Y = birth of young
2nd generation

prevents the development of
ave already shown, as has also
liver oil prevents the develop-
n. As nearly as we can judge
s furnished by Huldschinsky
healing at the cartilage-shaft
ultraviolet ray (or sunlight) is
hat which occurs after the
r oil, as determined by How-
me relations are also similar.
the ultraviolet ray produced
ng at the end of four weeks,
nths almost complete healing.
d that cod liver oil first gave
ng at the junctions of the car-
ng bones three weeks after the
, and that at the end of about
on of the diseased ends of the
lete. Moreover, as the result
ic examinations made on the
ucing diet, 3143, but exposed
to say that the changes pro-
skeleton do not differ in any
he changes produced when the
n light but on a diet supple-

upplied to rats living in room
ied with calcium but deficient
ng in fat-soluble A (and pos-
brings about an improvement
eneral. If the defects in the
to preclude the possibility of
all, cod liver oil promotes
pment and the storage of fat;
n of the hair and stimulates
productive power. The rat
approximates more nearly the
ous that cod liver oil does not
n. Its effect on the calcifica-
skeleton is merely the manifes-
single tissue.

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k, E. A.: Some Observations on Rickets, The Radiographic Evidence of the Influ-
ets, Bull. Johns Hopkins Hosp. 32: 314,

What we have just said in regard to cod liver oil, we may say, apparently with equal truth, in regard to sunlight. Sunlight, when supplied to the rats living on diets defective in their salt composition, as specified above, and in a factor or factors present in cod liver oil, brings about a general improvement in the condition of the animal. It promotes growth, muscular development, the accumulation of fat, the growth of the hair, sexual activity, and reproductive power. The rat becomes active, eats more food, and approximates closer the healthy animal. It has been no accident that sunlight has been found to exert a favorable effect in tuberculosis, in anemia, in the condition commonly referred to as the "exudative diathesis," in the treatment of surgical wounds and burns. Light cannot be thought of, any more than can cod liver oil, as acting on the skeleton alone. Though the deposition of lime salts which it causes affords a most striking, visible and measurable evidence of its action on the skeleton, its favorable influence there is probably not greater than its favorable influence elsewhere.

The experiments that we have reported have a biologic significance which extends far beyond the cure of rickets. Cod liver oil contains and light embodies something which is essential for optimal cellular function. Cod liver oil, or light, when made available to an animal previously deprived of them, permits the organism to put into successful operation mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defect in the composition of the diet directly by supplying to the body either calcium or phosphorus. Both must meet them indirectly, in a manner at present unknown, by so activating or altering the processes of the body as to secure a more efficient utilization of those substances which are directly or indirectly concerned with ossification and calcification. We do not believe that the diversity of favorable effects obtained from the therapeutic use of cod liver oil or of light can be explained in any other way than on the theory that by improving the efficiency of the organism it enables the organism to work with increased economy and to deal successfully with the exigencies of its environment.

Our experiments indicate that diets which are defective at room light or in darkness may cease to be defective in the presence of active light rays, and, conversely, that diets which are satisfactory in the sunlight may become unsatisfactory at room light or in darkness. It would seem that diets which suffice for the maintenance of optimal health during a life in darkness may supply an amount of dietary factors unnecessary for an individual who is irradiated by sunlight. From this point of view it becomes necessary to think of certain factors which can be taken into the body in the food as being able to compensate for deprivation of light, and of light as being able to compensate for the deprivation of certain dietary factors. The experiments suggest, therefore, that throughout the animal and perhaps also the vegetable kingdom there may be balances between the amounts of active light required and the amounts of certain food essentials requisite for the maintenance of health. Under the abnormal conditions imposed on animals by domestication or on man by civilization, it may be necessary to supply more light to replace the lack of certain dietary essentials or to increase the latter to compensate for deprivation of light. For example, the negro, because of his heavily pigmented skin absorbing light less readily than the white man, may require more light or, not receiving it, more of the corresponding factor or factors in the food, in order to maintain an optimal condition of health (e. g., to prevent the development of rickets). The available supply of light must be considered in the future in planning diets for prisoners, miners and all other individuals who are deprived of their exposure to light by occupation or other circumstances.

Our experiments indicate in a broader sense that just as we live in an atmosphere of air and breathe air, so we live in a medium of light and, so to speak, breathe light. That is, light, like air, is absorbed and in some way influences cellular function.

While one group of students of rickets has been groping for proof that the cause of the disease lay in physical conditions, another group has been too intent on the proofs of the curative properties of a dietary factor contained in cod liver oil for the proper consideration of other possibilities. Two factors, to all external appearances of a totally different nature, one

contained in cod liver oil and the other in light, are operative in the prevention and cure of rickets. This fact explains, in part at least, the confusion that has arisen concerning the etiology of the disease.

We do not regard the etiology of rickets as a solved problem. We do not doubt, however, that the demonstration of the curative action of light in rickets is a substantial step toward the complete understanding of the cause of this disease. Experiments with rats by Sherman and Pappenheimer[26] and ourselves[27] have shown that, in the absence of certain light rays and of a factor or factors contained in cod liver oil, a disease indistinguishable from the rickets of human beings may be produced through a deficiency of phosphates in the diet. Rickets so produced may be cured by cod liver oil or prevented by certain light rays. We have also shown that in rats fed on a diet in which the phosphate ion is sufficiently abundant, rickets does not develop even in the absence of certain light rays and of a factor or factors contained in cod liver oil.

Thus, experimentation with the rat makes it appear that deficiencies in light or in a factor or factors contained in cod liver oil do not cause rickets but rather permit defects in the inorganic composition of the diet to become effective. The disease is chiefly the expression in the skeleton of the disturbance of the inorganic metabolism in question. Our experiments have indicated that the function of light and of a factor or factors contained in cod liver oil, so far as rickets is concerned, is to exert a regulatory influence over the mineral metabolism of the body having to do with ossification and calcification. When the body is deprived of the regulatory action of certain light rays and of a factor or factors contained in cod liver oil, such disturbances in the inorganic metabolism of the body are permitted to develop.

As a result of these experiments with rats there is not the slightest doubt in our minds that either rickets or osteomalacia could be made to develop in human beings deprived of the protective influence of light rays and the dietary factor or factors contained in cod liver oil as easily as in the rat through similar alterations in the salt composition of the diet. Indeed, it may be that human beings are especially sensitive to even slight disturbances in the relations of the inorganic constituents of the diet when lacking the protective influence of light and the factor or factors contained in cod liver oil.

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[26] Sherman, H. C., and Pappenheimer, A. M.: Experimental Rickets in Rats, I, A Diet Producing Rickets in White Rats, and Its Prevention by the Addition of an Inorganic Salt, J. Exper. Med. **34:** 189 (Aug.) 1921.

[27] Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmonds, Nina: Studies on Experimental Rickets, III, A Pathological Condition Bearing Fundamental Resemblances to Rickets of the Human Being Resulting from Diets Low in Phosphorus and Fat-Soluble A: The Phosphate Ion in Its Prevention, Bull. Johns Hopkins Hosp. **32:** 160 (May) 1921.

Our experiments with the rat, therefore, make us think that in many instances the development of rickets in the human being may be due primarily to abnormal relations between certain mineral constituents of the diet; i. e., the disease has a purely exogenous origin. We are not at all certain, however, that the disease always has an exogenous origin. We think it probable, indeed, that rickets in some instances has an endogenous origin. It seems likely that in human beings, especially when deprived of the protective influence of certain light rays and of a factor or factors contained in cod liver oil, the mineral metabolism may be disorganized from causes operating within the body in such manner as to give rise to rickets.

SUMMARY

1. The object of the experiment was to determine whether or not sunlight prevents the development of rickets in the rat.

2. A diet was employed which at room light regularly gives rise to a disease in its essential features identical with rickets as seen in human beings. The diet was high in calcium, low in phosphorus and was insufficiently supplied with fat-soluble A. In other respects it was well constituted.

3. Eighteen rats were placed on the diet. Twelve were exposed to sunlight for a total of 242 hours over a period of sixty-two days. Six were kept under conditions of ordinary room light as control animals.

4. The control rats, killed with ether at the end of sixty days, all showed rickets.

5. The rats exposed to sunlight, killed coincidently, remained without exception entirely free from rickets. The absence of the lesions of rickets was confirmed by histologic examination.

lay and Watson,[19] on the etiology of rickets, Neve [20] called attention to the fact that rickets was exceedingly rare in Srinagar (India), where infants live under the worst conditions of hygiene and diet, but where sunlight, to which they are almost constantly exposed, abounds. He stated that the only case of rickets which he had seen there was in an English child. A cure took place when the gloomy house in which the patient lived was exchanged for one which was well lighted by the sun.

The favorable effect of sunlight in rickets as determined by clinical observation has been recently emphasized by Feer.[21] He called attention to the marked benefit which accrued in rickets from exposure to the sun's rays in the Swiss Alps. He made reference to the excellent results which had been obtained in the treatment of rickets with sunlight during the past ten years at the Zurich Sanatorium, and also to the investigation of Neumann [22] in 1909 in regard to the incidence of rickets and tetany in the Swiss Alps. Neumann had pointed out the great rarity of both rickets and tetany in Arosa, 1,740 meters (5,709 feet) above the sea level, and its frequency at Davos, at an altitude of 1,560 meters (4,285 feet) and only four and one-half hours distant. At Arosa the children lived for a large part of the time outdoors; but in Davos they lived largely indoors under the crowded conditions which are found generally in cities.

Huldschinsky made use of sunlight together with the ultraviolet ray in two cases of his series, and Riedel relied on treatment with sunlight in some of his cases, supplementing with the quartz lamp only on sunless days.

Hess and Unger [23] were the first, so far as we are aware, to demonstrate by means of the roentgen ray that sunlight alone possesses the same curative action as the light of the quartz lamp in the rickets of human beings. They state that they exposed five infants with rickets to the direct action of sunlight for periods vary-

ing from one-half hour to several hours daily, whenever the sunlight was available. Different parts of the body were in turn subjected to the action of the sun's rays. In one of the cases the patient was exposed to the sunlight only on seven occasions for a total period of twenty-five hours. The general condition of the infants, as well as the diseased condition in the bones,

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[19] Paton, D. N.; Findlay, L., and Watson, A.: Observations on the Cause of Rickets, Brit. M. J. **2**: 625 (Dec. 7) 1918.

[20] Neve, E. F.: The Etiology of Rickets, Brit. M. J **1**: 518, 1919.

[21] Feer, E.: Die Einwirkung des Höhenklimas auf das kranke Kind, Schweiz. med. Wchnschr. **51**: 438, 1921.

[22] Neumann, H.: Der Säugling im Hochgebirge, Deutsch. med. Wchnschr. **35**: 2167, 1909.

[23] Hess, A. F., and Unger, L. J.: The Cure of Infantile Rickets by Sunlight, J. A. M. A. **77**: 39 (July 2) 1921.

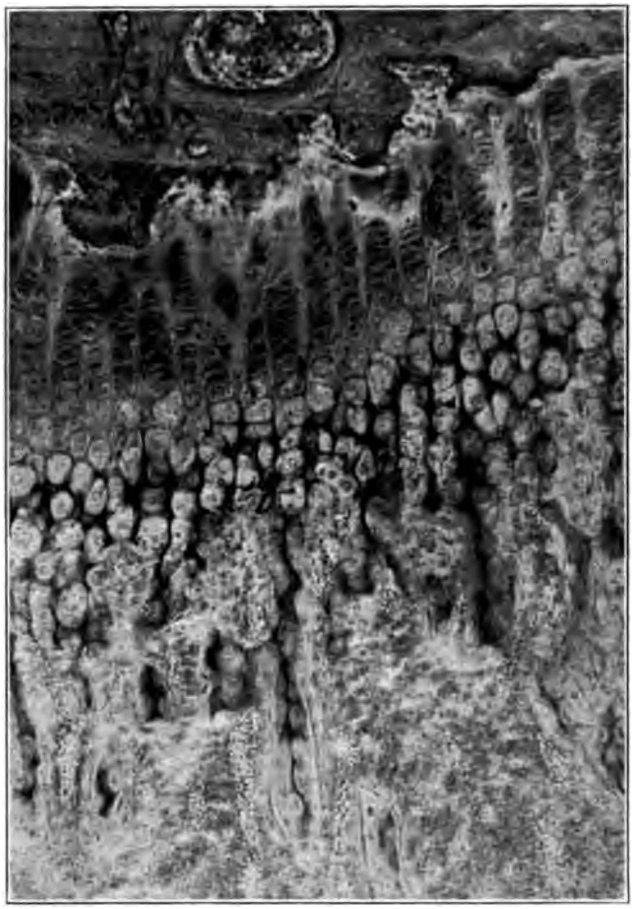

Fig. 4.—Epiphyseodiaphyseal junction of a bone from a rat on a rickets-producing diet, but exposed to sunlight. Note the complete calcification of cartilage and trabeculae.

was benefited. Calcification at the cartilage-shaft ju tions of the bones of the extremities occurring in t course of the treatment was determined by the roe gen ray.

EXPERIMENTAL: THE PREVENTION OF RICKETS THE RAT BY SUNLIGHT

All the investigations which have been made up the present time in regard to the curative effects of b the ultraviolet ray and sunlight in rickets have be made on human subjects of the disease, and all t evidence has been furnished by means of the roe genogram. In order to satisfy ourselves concerni the action of light in rickets as well as actually to s the changes produced in the bones, we performed th following experiments:

Eighteen rats about 6 weeks old and weighing between 40 and 50 gm. were placed on Diet 3143 which, as previous experience[24] has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings. The ration has the following composition: wheat, 33; maize, 33; gelatin, 15; wheat gluten, 15; sodium chlorid, 1; calcium carbonate, 3 per cent. It contains nearly twice the optimal content of calcium, and is decidedly below the optimum in its content of phosphorus and in fat-soluble A. Otherwise, it is well constituted.

Twelve rats placed on this diet were sent to New Haven, there to be exposed to sunlight. The remaining six rats were retained in Baltimore to be kept as control animals under ordinary laboratory conditions in a large, well ventilated room completely screened with windows of ordinary glass. The animals treated with the sunlight were divided into two groups and placed in fairly large wire mesh cages. Each clear day the cages were carried outdoors and placed in the sunlight. At first, the weather being warm, the rats, were exposed to the sunlight for two short periods of twenty minutes each. Soon, however, the periods were lengthened to six or even more hours. During the experimental period, which covered between sixty-two and

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[24] McCollum, E. V.; Simmonds, Nina; Shipley, P. G., and Park, E. A.: Studies on Experimental Rickets, VIII, The Production of Rickets by Diets Low in Phosphorus and Fat-Soluble A, J. Biol. Chem. **47**: 507, 1921.

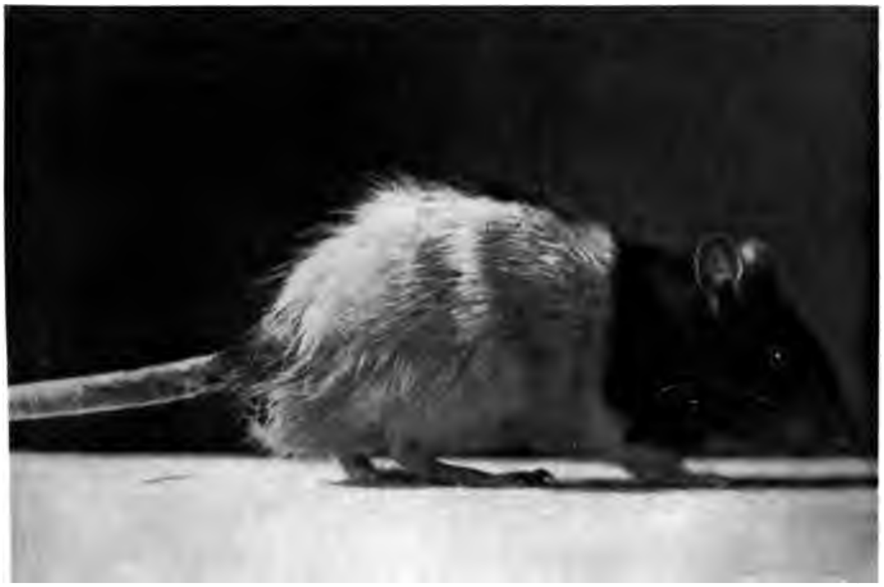

FIG. 1.

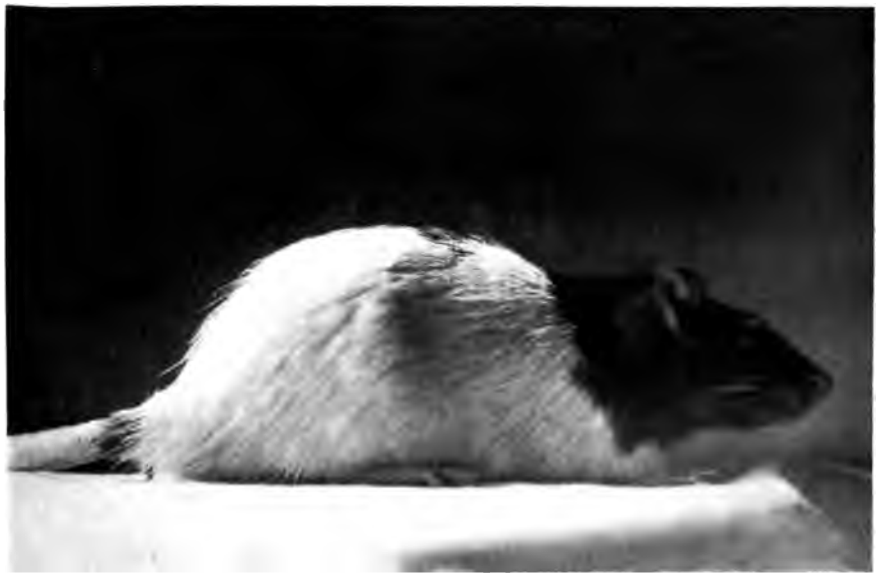

FIG. 2.

(McCollum, Simmonds, Shipley, and Park: Experimental rickets. XII.)

yellowish tint. Long before the experiments were completed, it became evident that the animals treated with sunlight were not developing rickets. Though they did not grow normally, they remained extremely active, climbing and darting about the cages. Toward the end of the experiments they became sexually active; one of the females became pregnant.

The control rats, killed at the expiration of two months, showed all the gross and microscopic evidences of rickets, the characteristic deformities of the thorax, enlargement and distortion of the costochondral junctions, fractures of the shafts, and enlargements at the wrists, ankles and knees, and the ends of all the long bones. The bones cut with diminished resistance. On section, a deep rachitic metaphysis entirely free from calcium was exposed. Into it the proliferative cartilage extended in irregular prolongations. The trabeculae were surrounded with broad zones of osteoid.

The rats exposed to the sunlight, on the other hand, showed none of the evidences of rickets. The thorax was not deformed; the costochondral junctions were normal. There were no fractures of the ribs. The ends of the long bones were not enlarged. The long bones cut with great resistance. On microscopic examination the cartilage was normal. The proliferative zone was completely calcified. The trabeculae were completely calcified. The condition found was normal, except that both microscopically and grossly the bone was more delicate than in the rat of corresponding age reared on satisfactory diets. Though the sunshine completely prevented the development of rickets, it did not entirely compensate for the deficiency of phosphorus in the diet, as regards the growth and development of the rat as a whole or of the skeleton.

There were some noteworthy findings outside the skeleton. An abundance of fat was present. In the control rats the fat was scant. The thymus was only partially involuted. The spleen was not enlarged.

The improvement in the general condition in our animals which were exposed to the light of the sun was convincing evidence that the effect of light was not on the skeleton alone. Sunlight obviously had a profound effect on every cell in the organism.

COMMENT

Sunlight effectually prevents the development of rickets in the rat. We have already shown, as has also Pappenheimer, that cod liver oil prevents the development of rickets in the rat. As nearly as we can judge from the roentgenograms furnished by Huldschinsky and others, the mode of healing at the cartilage-shaft junction induced by the ultraviolet ray (or sunlight) is exactly analogous to that which occurs after the administration of cod liver oil, as determined by Howland and Park.[25] The time relations are also similar. Huldschinsky found that the ultraviolet ray produced definite evidences of healing at the end of four weeks, and at the end of two months almost complete healing. Howland and Park found that cod liver oil first gave rise to evidences of healing at the junctions of the cartilage and shaft of the long bones three weeks after the administration was begun, and that at the end of about two months the calcification of the diseased ends of the shafts seemed to be complete. Moreover, as the result of the gross and histologic examinations made on the rats fed the rickets-producing diet, 3143, but exposed to sunlight, it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod liver oil.

When cod liver oil is supplied to rats living in room light on diets well supplied with calcium but deficient in phosphorus and lacking in fat-soluble A (and possibly another factor), it brings about an improvement in the animal which is general. If the defects in the diet are not so severe as to preclude the possibility of any favorable effect at all, cod liver oil promotes growth, muscular development and the storage of fat; it improves the condition of the hair and stimulates sexual activity and reproductive power. The rat becomes more active and approximates more nearly the normal animal. It is obvious that cod liver oil does not act on the skeleton alone. Its effect on the calcification and growth of the skeleton is merely the manifestation of its effect on a single tissue.

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[25] Howland, John, and Park, E. A.: Some Observations on Rickets, Arch. Pediat. **37**: 411, 1920: The Radiographic Evidence of the Influence of Cod Liver Oil in Rickets, Bull. Johns Hopkins Hosp. **32**: 314, 1921.

What we have just said in regard to cod liver oil, we may say, apparently with equal truth, in regard to sunlight. Sunlight, when supplied to the rats living on diets defective in their salt composition, as specified above, and in a factor or factors present in cod liver oil, brings about a general improvement in the condition of the animal. It promotes growth, muscular development, the accumulation of fat, the growth of the hair, sexual activity, and reproductive power. The rat becomes active, eats more food, and approximates closer the healthy animal. It has been no accident that sunlight has been found to exert a favorable effect in tuberculosis, in anemia, in the condition commonly referred to as the "exudative diathesis," in the treatment of surgical wounds and burns. Light cannot be thought of, any more than can cod liver oil, as acting on the skeleton alone. Though the deposition of lime salts which it causes affords a most striking, visible and measurable evidence of its action on the skeleton, its favorable influence there is probably not greater than its favorable influence elsewhere.

The experiments that we have reported have a biologic significance which extends far beyond the cure of rickets. Cod liver oil contains and light embodies something which is essential for optimal cellular function. Cod liver oil, or light, when made available to an animal previously deprived of them, permits the organism to put into successful operation mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defect in the composition of the diet directly by supplying to the body either calcium or phosphorus. Both must meet them indirectly, in a manner at present unknown, by so activating or altering the processes of the body as to secure a more efficient utilization of those substances which are directly or indirectly concerned with ossification and calcification. We do not believe that the diversity of favorable effects obtained from the therapeutic use of cod liver oil or of light can be explained in any other way than on the theory that by improving the efficiency of the organism it enables the organism to work with increased economy and to deal successfully with the exigencies of its environment.

Our experiments indicate that diets which are defective at room light or in darkness may cease to be defective in the presence of active light rays, and, conversely, that diets which are satisfactory in the sunlight may become unsatisfactory at room light or in darkness. It would seem that diets which suffice for the maintenance of optimal health during a life in darkness may supply an amount of dietary factors unnecessary for an individual who is irradiated by sunlight. From this point of view it becomes necessary to think of certain factors which can be taken into the body in the food as being able to compensate for deprivation of light, and of light as being able to compensate for the deprivation of certain dietary factors. The experiments suggest, therefore, that throughout the animal and perhaps also the vegetable kingdom there may be balances between the amounts of active light required and the amounts of certain food essentials requisite for the maintenance of health. Under the abnormal conditions imposed on animals by domestication or on man by civilization, it may be necessary to supply more light to replace the lack of certain dietary essentials or to increase the latter to compensate for deprivation of light. For example, the negro, because of his heavily pigmented skin absorbing light less readily than the white man, may require more light or, not receiving it, more of the corresponding factor or factors in the food, in order to maintain an optimal condition of health (e. g., to prevent the development of rickets). The available supply of light must be considered in the future in planning diets for prisoners, miners and all other individuals who are deprived of their exposure to light by occupation or other circumstances.

Our experiments indicate in a broader sense that just as we live in an atmosphere of air and breathe air, so we live in a medium of light and, so to speak, breathe light. That is, light, like air, is absorbed and in some way influences cellular function.

While one group of students of rickets has been groping for proof that the cause of the disease lay in physical conditions, another group has been too intent on the proofs of the curative properties of a dietary factor contained in cod liver oil for the proper consideration of other possibilities. Two factors, to all external appearances of a totally different nature, one

reveals unsuspected dirt, but is Nature's universal disinfectant, as well as a stimulant and tonic. Such knowledge will also stimulate efforts for the abatement of smoke, and for the multiplication of open spaces, especially as playgrounds for the children of the poor.

Experimental evidence for the favorable action of sunlight on the mineral metabolism was furnished by Raczynski [18] in 1912. On account of its importance, we quote Raczynski's brief communication in its entirety:

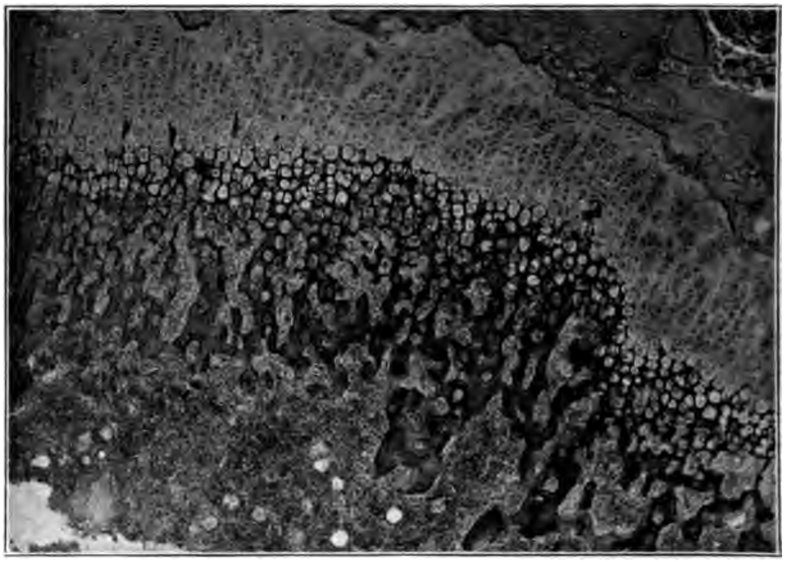

Fig. 3.—Section from a long bone of a rat which had been on the same diet (3143) as the control shown in Figure 1. The cartilage is narrow, regular and well calcified. The marrow is normal, and the bony trabeculae of the shaft, while somewhat fewer than those of a normal bone, are well calcified and show evidences of normal osteoblastic activity. This animal was exposed to sunlight in the manner described in the test.

There are numerous investigations which have had as their object the determination of the etiology of rickets, but there exists none which consists in an experimental inquiry into the action of the sun in that disease.

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[18] Raczynski, J.: Communications sur le rachitisme: I, Recherches experimentales sur le manque d'action du soleil comme cause du rachitisme, Compt. rend. de l'Association internationale de pédiatrie, 1912, p. 308.

Daily experience and statistics show that the greatest number of cases of rickets are found in the months following the winter, i. e., in March, April and May.

The statistical records of rachitic breast fed infants aged between 3 and 11 months appearing at the children's outpatient department at Leopol give a curve which begins to ascend in January, rises sharply in the months following, and attains its height in May, to fall rapidly in June.

The attempt is made to explain these generally recognized facts by improper hygienic conditions, such as impure air and damp, ill ventilated lodgings in which the infants of the poor live during the winter. But we often encounter rachitic children in the same months of the year among our patients who live under the most favorable hygienic conditions.

There are again many other facts which lead us to say that it is the sun which plays the principal rôle in the etiology of rickets.

The following experiment lends support to our thesis:

Two puppies born of the same mother in May were reared for six weeks, the first in the sunlight from morning to evening, the second in absolute darkness in a large, well ventilated cage. Both were nourished in the same manner, that is to say, they were both exclusively suckled by their mother. At the end of six weeks the puppies were killed and their bodies were examined from the chemical standpoint.

I have looked for Ca, P, Mg, Cl and Fe, and have estimated them per 100 grams of body weight:

	In the Case of the Dog Reared in Sunlight Gm.	In the Case of the Dog Reared in Darkness Gm.
CaO ...	1.578	0.978
P_2O_5 ...	1.192	0.862
MgO ..	0.054	0.041
Cl	0.162	0.347
Fe	0.017	0.019

The table shows that the body of the dog reared in the darkness contained less CaO and P_2O_5 than the body of the dog reared in the sunlight; and that a diminution of CaO and P_2O_5 in the organism is a characteristic evidence of rickets.

It is possible to assume that the lack of the action of the sunlight by influencing in so unfavorable a manner the assimilation of CaO in the young organism is one of the causes of rickets. This is in complete accord with clinical experience.

In a recent letter to the *British Medical Journal* in criticism of the conclusions arrived at by Paton, Find-

lay and Watson,[19] on the etiology of rickets, Neve [20] called attention to the fact that rickets was exceedingly rare in Srinagar (India), where infants live under the worst conditions of hygiene and diet, but where sunlight, to which they are almost constantly exposed, abounds. He stated that the only case of rickets which he had seen there was in an English child. A cure took place when the gloomy house in which the patient lived was exchanged for one which was well lighted by the sun.

The favorable effect of sunlight in rickets as determined by clinical observation has been recently emphasized by Feer.[21] He called attention to the marked benefit which accrued in rickets from exposure to the sun's rays in the Swiss Alps. He made reference to the excellent results which had been obtained in the treatment of rickets with sunlight during the past ten years at the Zurich Sanatorium, and also to the investigation of Neumann [22] in 1909 in regard to the incidence of rickets and tetany in the Swiss Alps. Neumann had pointed out the great rarity of both rickets and tetany in Arosa, 1,740 meters (5,709 feet) above the sea level, and its frequency at Davos, at an altitude of 1,560 meters (4,285 feet) and only four and one-half hours distant. At Arosa the children lived for a large part of the time outdoors; but in Davos they lived largely indoors under the crowded conditions which are found generally in cities.

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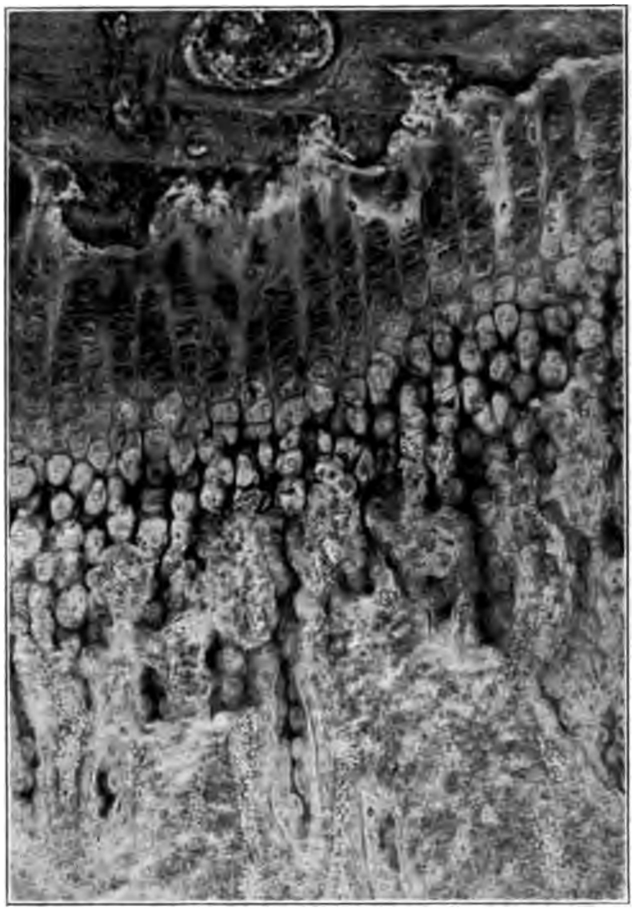

Fig. 4.—Epiphyseodiaphyseal junction of a bone from a rat on a rickets-producing diet, but exposed to sunlight. Note the complete calcification of cartilage and trabeculae.

THE PREVENTION OF THE DEVELOPMENT OF RICKETS IN RATS BY SUNLIGHT

STUDIES ON EXPERIMENTAL RICKETS: XIV *

G. F. POWERS, M.D., AND E. A. PARK, M.D.,
Assistant Professor and Professor in Pediatrics, Respectively, Yale University School of Medicine
NEW HAVEN, CONN.

P. G. SHIPLEY, M.D.
Instructor in Pediatrics, Johns Hopkins University Medical Department
AND
E. V. McCOLLUM, PH.D., AND NINA SIMMONDS, S.B.
Professor of Biochemistry and Associate in Biochemistry, Respectively, Department of Chemical Hygiene, Johns Hopkins University School of Hygiene and Public Health
BALTIMORE

HISTORICAL: LIGHT IN THE CURE OF RICKETS AND TETANY

In 1904, Buchholz[1] published a paper on the treatment by means of light of rickets and certain other diseases occurring in children. He used the "Glühlicht" (Kellogg) which, he stated, was poor in chemically active rays. The heat rays were in part absorbed by a special kind of glass filter interposed between lamp and patient. He considered that the action of the lamp depended in part on the heat rays, in part on the light rays. Whatever the exact composition of the light may have been, Buchholz reported favorable results from its use in rickets. Altogether he treated with it sixteen rachitic children. They all showed general improvement. They ate and slept better; they became more active and began to walk. Sweating of the head decreased, and their backs became straight. The work of Buchholz seems to have commanded little, if any, attention.

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* Read before the American Child Hygiene Association at the Twelfth Annual Meeting at New Haven, Conn., Nov. 2-5, 1921.

[1] Buchholz, E.: Ueber Lichtbehandlung der Rachitis und anderer Kinderkrankheiten, Verhandlungen der Gesellschaft für Kinderheilkunde in der Abteilung für Kinderheilkunde der 76 Versammlung der Gesellschaft Deutscher Naturforscher und Aerzte in Breslau, **21**: 116, 1904.

yellowish tint. Long before the experiments were completed, it became evident that the animals treated with sunlight were not developing rickets. Though they did not grow normally, they remained extremely active, climbing and darting about the cages. Toward the end of the experiments they became sexually active; one of the females became pregnant.

The control rats, killed at the expiration of two months, showed all the gross and microscopic evidences of rickets, the characteristic deformities of the thorax, enlargement and distortion of the costochondral junctions, fractures of the shafts, and enlargements at the wrists, ankles and knees, and the ends of all the long bones. The bones cut with diminished resistance. On section, a deep rachitic metaphysis entirely free from calcium was exposed. Into it the proliferative cartilage extended in irregular prolongations. The trabeculae were surrounded with broad zones of osteoid.

The rats exposed to the sunlight, on the other hand, showed none of the evidences of rickets. The thorax was not deformed; the costochondral junctions were normal. There were no fractures of the ribs. The ends of the long bones were not enlarged. The long bones cut with great resistance. On microscopic examination the cartilage was normal. The proliferative zone was completely calcified. The trabeculae were completely calcified. The condition found was normal, except that both microscopically and grossly the bone was more delicate than in the rat of corresponding age reared on satisfactory diets. Though the sunshine completely prevented the development of rickets, it did not entirely compensate for the deficiency of phosphorus in the diet, as regards the growth and development of the rat as a whole or of the skeleton.

There were some noteworthy findings outside the skeleton. An abundance of fat was present. In the control rats the fat was scant. The thymus was only partially involuted. The spleen was not enlarged.

The improvement in the general condition in our animals which were exposed to the light of the sun was convincing evidence that the effect of light was not on the skeleton alone. Sunlight obviously had a profound effect on every cell in the organism.

COMMENT

Sunlight effectually prevents the development of rickets in the rat. We have already shown, as has also Pappenheimer, that cod liver oil prevents the development of rickets in the rat. As nearly as we can judge from the roentgenograms furnished by Huldschinsky and others, the mode of healing at the cartilage-shaft junction induced by the ultraviolet ray (or sunlight) is exactly analogous to that which occurs after the administration of cod liver oil, as determined by Howland and Park.[25] The time relations are also similar. Huldschinsky found that the ultraviolet ray produced definite evidences of healing at the end of four weeks, and at the end of two months almost complete healing. Howland and Park found that cod liver oil first gave rise to evidences of healing at the junctions of the cartilage and shaft of the long bones three weeks after the administration was begun, and that at the end of about two months the calcification of the diseased ends of the shafts seemed to be complete. Moreover, as the result of the gross and histologic examinations made on the rats fed the rickets-producing diet, 3143, but exposed to sunlight, it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod liver oil.

When cod liver oil is supplied to rats living in room light on diets well supplied with calcium but deficient in phosphorus and lacking in fat-soluble A (and possibly another factor), it brings about an improvement in the animal which is general. If the defects in the diet are not so severe as to preclude the possibility of any favorable effect at all, cod liver oil promotes growth, muscular development and the storage of fat; it improves the condition of the hair and stimulates sexual activity and reproductive power. The rat becomes more active and approximates more nearly the normal animal. It is obvious that cod liver oil does not act on the skeleton alone. Its effect on the calcification and growth of the skeleton is merely the manifestation of its effect on a single tissue.

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[25] Howland, John, and Park, E. A.: Some Observations on Rickets, Arch. Pediat. **37**: 411, 1920: The Radiographic Evidence of the Influence of Cod Liver Oil in Rickets, Bull. Johns Hopkins Hosp. **32**: 314, 1921.

What we have just said in regard to cod liver oil, we may say, apparently with equal truth, in regard to sunlight. Sunlight, when supplied to the rats living on diets defective in their salt composition, as specified above, and in a factor or factors present in cod liver oil, brings about a general improvement in the condition of the animal. It promotes growth, muscular development, the accumulation of fat, the growth of the hair, sexual activity, and reproductive power. The rat becomes active, eats more food, and approximates closer the healthy animal. It has been no accident that sunlight has been found to exert a favorable effect in tuberculosis, in anemia, in the condition commonly referred to as the "exudative diathesis," in the treatment of surgical wounds and burns. Light cannot be thought of, any more than can cod liver oil, as acting on the skeleton alone. Though the deposition of lime salts which it causes affords a most striking, visible and measurable evidence of its action on the skeleton, its favorable influence there is probably not greater than its favorable influence elsewhere.

The experiments that we have reported have a biologic significance which extends far beyond the cure of rickets. Cod liver oil contains and light embodies something which is essential for optimal cellular function. Cod liver oil, or light, when made available to an animal previously deprived of them, permits the organism to put into successful operation mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defect in the composition of the diet directly by supplying to the body either calcium or phosphorus. Both must meet them indirectly, in a manner at present unknown, by so activating or altering the processes of the body as to secure a more efficient utilization of those substances which are directly or indirectly concerned with ossification and calcification. We do not believe that the diversity of favorable effects obtained from the therapeutic use of cod liver oil or of light can be explained in any other way than on the theory that by improving the efficiency of the organism it enables the organism to work with increased economy and to deal successfully with the exigencies of its environment.

Our experiments indicate that diets which are defective at room light or in darkness may cease to be defective in the presence of active light rays, and, conversely, that diets which are satisfactory in the sunlight may become unsatisfactory at room light or in darkness. It would seem that diets which suffice for the maintenance of optimal health during a life in darkness may supply an amount of dietary factors unnecessary for an individual who is irradiated by sunlight. From this point of view it becomes necessary to think of certain factors which can be taken into the body in the food as being able to compensate for deprivation of light, and of light as being able to compensate for the deprivation of certain dietary factors. The experiments suggest, therefore, that throughout the animal and perhaps also the vegetable kingdom there may be balances between the amounts of active light required and the amounts of certain food essentials requisite for the maintenance of health. Under the abnormal conditions imposed on animals by domestication or on man by civilization, it may be necessary to supply more light to replace the lack of certain dietary essentials or to increase the latter to compensate for deprivation of light. For example, the negro, because of his heavily pigmented skin absorbing light less readily than the white man, may require more light or, not receiving it, more of the corresponding factor or factors in the food, in order to maintain an optimal condition of health (e. g., to prevent the development of rickets). The available supply of light must be considered in the future in planning diets for prisoners, miners and all other individuals who are deprived of their exposure to light by occupation or other circumstances.

Our experiments indicate in a broader sense that just as we live in an atmosphere of air and breathe air, so we live in a medium of light and, so to speak, breathe light. That is, light, like air, is absorbed and in some way influences cellular function.

While one group of students of rickets has been groping for proof that the cause of the disease lay in physical conditions, another group has been too intent on the proofs of the curative properties of a dietary factor contained in cod liver oil for the proper consideration of other possibilities. Two factors, to all external appearances of a totally different nature, one

reveals unsuspected dirt, but is Nature's universal disinfectant, as well as a stimulant and tonic. Such knowledge will also stimulate efforts for the abatement of smoke, and for the multiplication of open spaces, especially as playgrounds for the children of the poor.

Experimental evidence for the favorable action of sunlight on the mineral metabolism was furnished by Raczynski [18] in 1912. On account of its importance, we quote Raczynski's brief communication in its entirety:

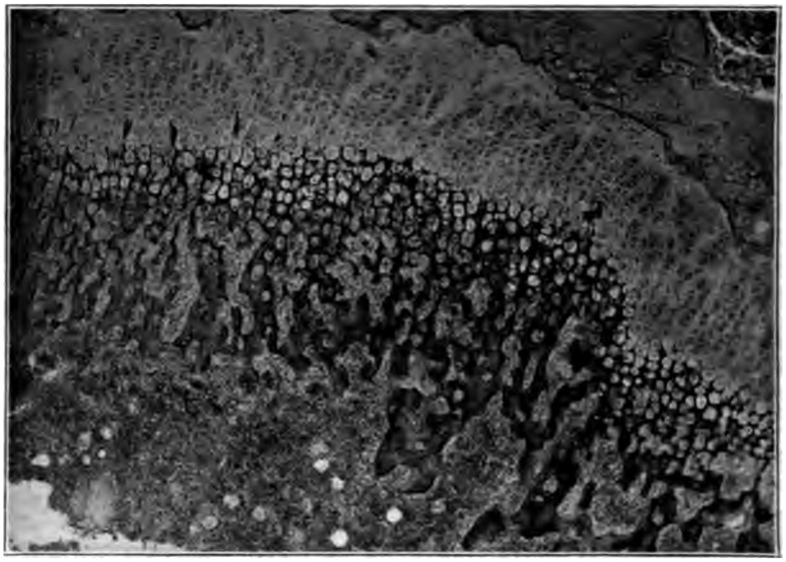

Fig. 3.—Section from a long bone of a rat which had been on the same diet (3143) as the control shown in Figure 1. The cartilage is narrow, regular and well calcified. The marrow is normal, and the bony trabeculae of the shaft, while somewhat fewer than those of a normal bone, are well calcified and show evidences of normal osteoblastic activity. This animal was exposed to sunlight in the manner described in the test.

There are numerous investigations which have had as their object the determination of the etiology of rickets, but there exists none which consists in an experimental inquiry into the action of the sun in that disease.

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[18] Raczynski, J.: Communications sur le rachitisme: I, Recherches experimentales sur le manque d'action du soleil comme cause du rachitisme, Compt. rend. de l'Association internationale de pédiatrie, 1912, p. 308.

Daily experience and statistics show that the greatest number of cases of rickets are found in the months following the winter, i. e., in March, April and May.

The statistical records of rachitic breast fed infants aged between 3 and 11 months appearing at the children's outpatient department at Leopol give a curve which begins to ascend in January, rises sharply in the months following, and attains its height in May, to fall rapidly in June.

The attempt is made to explain these generally recognized facts by improper hygienic conditions, such as impure air and damp, ill ventilated lodgings in which the infants of the poor live during the winter. But we often encounter rachitic children in the same months of the year among our patients who live under the most favorable hygienic conditions.

There are again many other facts which lead us to say that it is the sun which plays the principal rôle in the etiology of rickets.

The following experiment lends support to our thesis:

Two puppies born of the same mother in May were reared for six weeks, the first in the sunlight from morning to evening, the second in absolute darkness in a large, well ventilated cage. Both were nourished in the same manner, that is to say, they were both exclusively suckled by their mother. At the end of six weeks the puppies were killed and their bodies were examined from the chemical standpoint.

I have looked for Ca, P, Mg, Cl and Fe, and have estimated them per 100 grams of body weight:

	In the Case of the Dog Reared in Sunlight Gm.	In the Case of the Dog Reared in Darkness Gm.
CaO ...	1.578	0.978
P_2O_5 ...	1.192	0.862
MgO ..	0.054	0.041
Cl	0.162	0.347
Fe	0.017	0.019

The table shows that the body of the dog reared in the darkness contained less CaO and P_2O_5 than the body of the dog reared in the sunlight; and that a diminution of CaO and P_2O_5 in the organism is a characteristic evidence of rickets.

It is possible to assume that the lack of the action of the sunlight by influencing in so unfavorable a manner the assimilation of CaO in the young organism is one of the causes of rickets. This is in complete accord with clinical experience.

In a recent letter to the *British Medical Journal* in criticism of the conclusions arrived at by Paton, Findlay and Watson,[19] on the etiology of rickets, Neve[20] called attention to the fact that rickets was exceedingly rare in Srinagar (India), where infants live under the worst conditions of hygiene and diet, but where sunlight, to which they are almost constantly exposed, abounds. He stated that the only case of rickets which he had seen there was in an English child. A cure took place when the gloomy house in which the patient lived was exchanged for one which was well lighted by the sun.

The favorable effect of sunlight in rickets as determined by clinical observation has been recently emphasized by Feer.[21] He called attention to the marked benefit which accrued in rickets from exposure to the sun's rays in the Swiss Alps. He made reference to the excellent results which had been obtained in the treatment of rickets with sunlight during the past ten years at the Zurich Sanatorium, and also to the investigation of Neumann[22] in 1909 in regard to the incidence of rickets and tetany in the Swiss Alps. Neumann had pointed out the great rarity of both rickets and tetany in Arosa, 1,740 meters (5,709 feet) above the sea level, and its frequency at Davos, at an altitude of 1,560 meters (4,285 feet) and only four and one-half hours distant. At Arosa the children lived for a large part of the time outdoors; but in Davos they lived largely indoors under the crowded conditions which are found generally in cities.

Huldschinsky made use of sunlight together with the ultraviolet ray in two cases of his series, and Riedel relied on treatment with sunlight in some of his cases, supplementing with the quartz lamp only on sunless days.

Hess and Unger[23] were the first, so far as we are aware, to demonstrate by means of the roentgen ray that sunlight alone possesses the same curative action as the light of the quartz lamp in the rickets of human beings. They state that they exposed five infants with rickets to the direct action of sunlight for periods vary-

ing from one-half hour to several hours daily, whenever the sunlight was available. Different parts of the body were in turn subjected to the action of the sun's rays. In one of the cases the patient was exposed to the sunlight only on seven occasions for a total period of twenty-five hours. The general condition of the infants, as well as the diseased condition in the bones,

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[19] Paton, D. N.; Findlay, L., and Watson, A.: Observations on the Cause of Rickets, Brit. M. J. **2**: 625 (Dec. 7) 1918.

[20] Neve, E. F.: The Etiology of Rickets, Brit. M. J **1**: 518, 1919.

[21] Feer, E.: Die Einwirkung des Höhenklimas auf das kranke Kind, Schweiz. med. Wchnschr. **51**: 438, 1921.

[22] Neumann, H.: Der Säugling im Hochgebirge, Deutsch. med. Wchnschr. **35**: 2167, 1909.

[23] Hess, A. F., and Unger, L. J.: The Cure of Infantile Rickets by Sunlight, J. A. M. A. **77**: 39 (July 2) 1921.

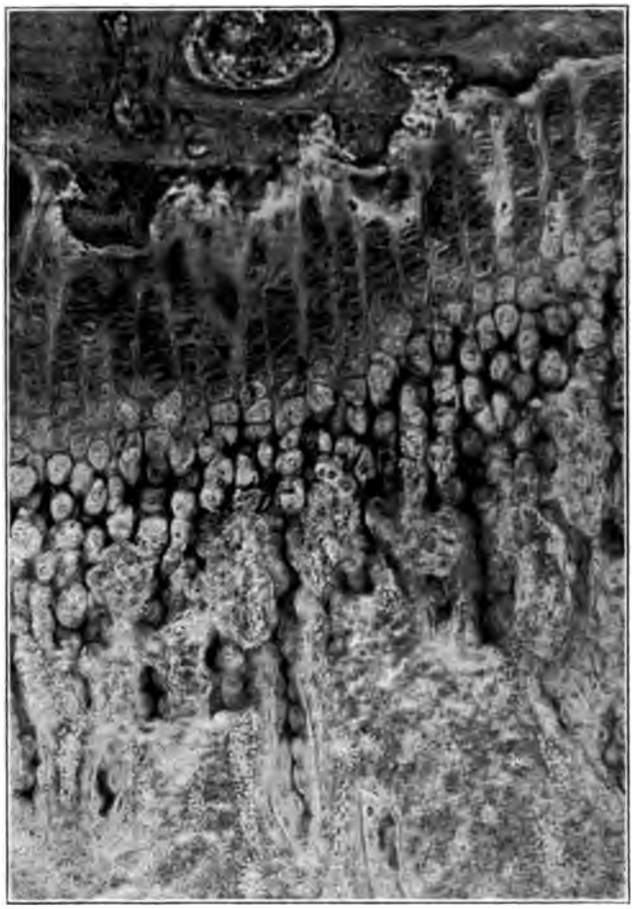

Fig. 4.—Epiphyseodiaphyseal junction of a bone from a rat on a rickets-producing diet, but exposed to sunlight. Note the complete calcification of cartilage and trabeculae.

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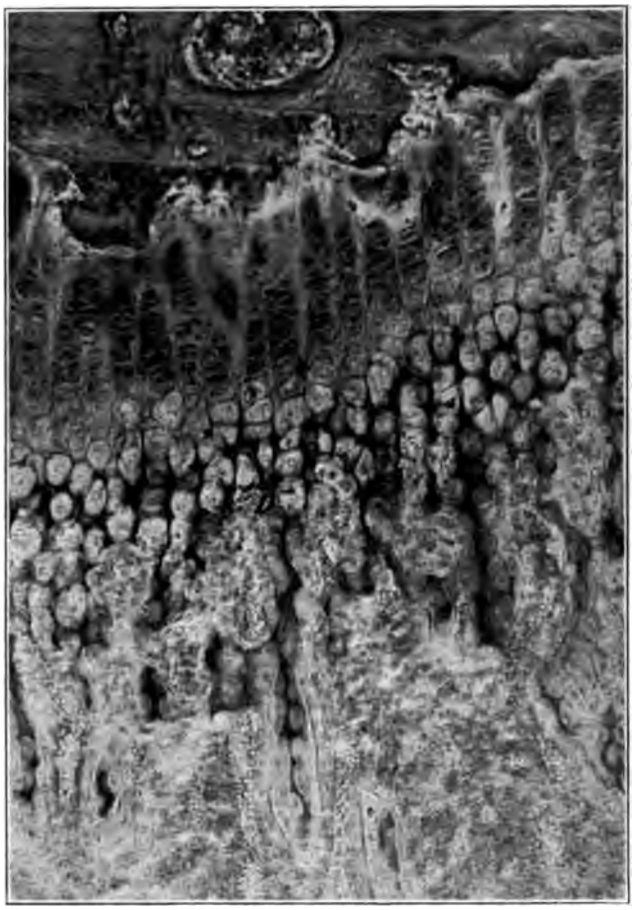

Fig. 4.—Epiphyseodiaphyseal junction of a bone from a rat on a rickets-producing diet, but exposed to sunlight. Note the complete calcification of cartilage and trabeculae.

was benefited. Calcification at the cartilage-shaft junctions of the bones of the extremities occurring in the course of the treatment was determined by the roentgen ray.

EXPERIMENTAL: THE PREVENTION OF RICKETS IN THE RAT BY SUNLIGHT

All the investigations which have been made up to the present time in regard to the curative effects of both the ultraviolet ray and sunlight in rickets have been made on human subjects of the disease, and all the evidence has been furnished by means of the roentgenogram. In order to satisfy ourselves concerning the action of light in rickets as well as actually to see the changes produced in the bones, we performed the following experiments:

Eighteen rats about 6 weeks old and weighing between 40 and 50 gm. were placed on Diet 3143 which, as previous experience [24] has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings. The ration has the following composition: wheat, 33; maize, 33; gelatin, 15; wheat gluten, 15; sodium chlorid, 1; calcium carbonate, 3 per cent. It contains nearly twice the optimal content of calcium, and is decidedly below the optimum in its content of phosphorus and in fat-soluble A. Otherwise, it is well constituted.

Twelve rats placed on this diet were sent to New Haven, there to be exposed to sunlight. The remaining six rats were retained in Baltimore to be kept as control animals under ordinary laboratory conditions in a large, well ventilated room completely screened with windows of ordinary glass. The animals treated with the sunlight were divided into two groups and placed in fairly large wire mesh cages. Each clear day the cages were carried outdoors and placed in the sunlight. At first, the weather being warm, the rats, were exposed to the sunlight for two short periods of twenty minutes each. Soon, however, the periods were lengthened to six or even more hours. During the experimental period, which covered between sixty-two and sixty-seven days, the rats were exposed to the sunlight on every day except nine. The total exposure to sunlight varied between 242 and 273 hours. The average daily exposure was four hours.

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[24] McCollum, E. V.; Simmonds, Nina; Shipley, P. G., and Park, E. A.: Studies on Experimental Rickets, VIII, The Production of Rickets by Diets Low in Phosphorus and Fat-Soluble A, J. Biol. Chem. **47**: 507, 1921.

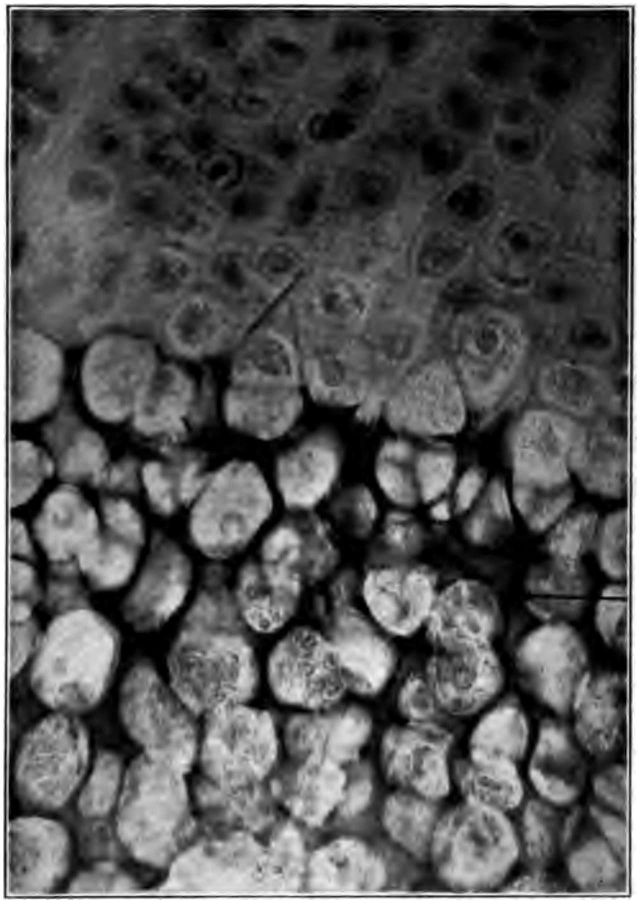

Fig. 5.—Highly magnified cartilage from the provisional zone of calcification of the bone of a rat exposed to sunlight while on a rickets-producing diet. This picture shows the deposit of lime salts between the cartilage cells (indicated by arrow).

When first exposed to sunlight, the albinos developed conjunctivitis; the ears of all, in particular the albinos, began to peel; the skin of the tails became sunburned and rough; the hair of some of the albinos acquired a

yellowish tint. Long before the experiments were completed, it became evident that the animals treated with sunlight were not developing rickets. Though they did not grow normally, they remained extremely active, climbing and darting about the cages. Toward the end of the experiments they became sexually active; one of the females became pregnant.

The control rats, killed at the expiration of two months, showed all the gross and microscopic evidences of rickets, the characteristic deformities of the thorax, enlargement and distortion of the costochondral junctions, fractures of the shafts, and enlargements at the wrists, ankles and knees, and the ends of all the long bones. The bones cut with diminished resistance. On section, a deep rachitic metaphysis entirely free from calcium was exposed. Into it the proliferative cartilage extended in irregular prolongations. The trabeculae were surrounded with broad zones of osteoid.

The rats exposed to the sunlight, on the other hand, showed none of the evidences of rickets. The thorax was not deformed; the costochondral junctions were normal. There were no fractures of the ribs. The ends of the long bones were not enlarged. The long bones cut with great resistance. On microscopic examination the cartilage was normal. The proliferative zone was completely calcified. The trabeculae were completely calcified. The condition found was normal, except that both microscopically and grossly the bone was more delicate than in the rat of corresponding age reared on satisfactory diets. Though the sunshine completely prevented the development of rickets, it did not entirely compensate for the deficiency of phosphorus in the diet, as regards the growth and development of the rat as a whole or of the skeleton.

There were some noteworthy findings outside the skeleton. An abundance of fat was present. In the control rats the fat was scant. The thymus was only partially involuted. The spleen was not enlarged.

The improvement in the general condition in our animals which were exposed to the light of the sun was convincing evidence that the effect of light was not on the skeleton alone. Sunlight obviously had a profound effect on every cell in the organism.

COMMENT

Sunlight effectually prevents the development of rickets in the rat. We have already shown, as has also Pappenheimer, that cod liver oil prevents the development of rickets in the rat. As nearly as we can judge from the roentgenograms furnished by Huldschinsky and others, the mode of healing at the cartilage-shaft junction induced by the ultraviolet ray (or sunlight) is exactly analogous to that which occurs after the administration of cod liver oil, as determined by Howland and Park.[25] The time relations are also similar. Huldschinsky found that the ultraviolet ray produced definite evidences of healing at the end of four weeks, and at the end of two months almost complete healing. Howland and Park found that cod liver oil first gave rise to evidences of healing at the junctions of the cartilage and shaft of the long bones three weeks after the administration was begun, and that at the end of about two months the calcification of the diseased ends of the shafts seemed to be complete. Moreover, as the result of the gross and histologic examinations made on the rats fed the rickets-producing diet, 3143, but exposed to sunlight, it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod liver oil.

When cod liver oil is supplied to rats living in room light on diets well supplied with calcium but deficient in phosphorus and lacking in fat-soluble A (and possibly another factor), it brings about an improvement in the animal which is general. If the defects in the diet are not so severe as to preclude the possibility of any favorable effect at all, cod liver oil promotes growth, muscular development and the storage of fat; it improves the condition of the hair and stimulates sexual activity and reproductive power. The rat becomes more active and approximates more nearly the normal animal. It is obvious that cod liver oil does not act on the skeleton alone. Its effect on the calcification and growth of the skeleton is merely the manifestation of its effect on a single tissue.

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25. Howland, John, and Park, E. A.: Some Observations on Rickets, Arch. Pediat. **37**: 411, 1920: The Radiographic Evidence of the Influence of Cod Liver Oil in Rickets, Bull. Johns Hopkins Hosp. **32**: 314, 1921.

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The experiments that we have reported have a biologic significance which extends far beyond the cure of rickets. Cod liver oil contains and light embodies something which is essential for optimal cellular function. Cod liver oil, or light, when made available to an animal previously deprived of them, permits the organism to put into successful operation mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defect in the composition of the diet directly by supplying to the body either calcium or phosphorus. Both must meet them indirectly, in a manner at present unknown, by so activating or altering the processes of the body as to secure a more efficient utilization of those substances which are directly or indirectly concerned with ossification and calcification. We do not believe that the diversity of favorable effects obtained from the therapeutic use of cod liver oil or of light can be explained in any other way than on the theory that by improving the efficiency of the organism it enables the organism to work with increased economy and to deal successfully with the exigencies of its environment.

Our experiments indicate that diets which are defective at room light or in darkness may cease to be defective in the presence of active light rays, and, conversely, that diets which are satisfactory in the sunlight may become unsatisfactory at room light or in darkness. It would seem that diets which suffice for the maintenance of optimal health during a life in darkness may supply an amount of dietary factors unnecessary for an individual who is irradiated by sunlight. From this point of view it becomes necessary to think of certain factors which can be taken into the body in the food as being able to compensate for deprivation of light, and of light as being able to compensate for the deprivation of certain dietary factors. The experiments suggest, therefore, that throughout the animal and perhaps also the vegetable kingdom there may be balances between the amounts of active light required and the amounts of certain food essentials requisite for the maintenance of health. Under the abnormal conditions imposed on animals by domestication or on man by civilization, it may be necessary to supply more light to replace the lack of certain dietary essentials or to increase the latter to compensate for deprivation of light. For example, the negro, because of his heavily pigmented skin absorbing light less readily than the white man, may require more light or, not receiving it, more of the corresponding factor or factors in the food, in order to maintain an optimal condition of health (e. g., to prevent the development of rickets). The available supply of light must be considered in the future in planning diets for prisoners, miners and all other individuals who are deprived of their exposure to light by occupation or other circumstances.

Our experiments indicate in a broader sense that just as we live in an atmosphere of air and breathe air, so we live in a medium of light and, so to speak, breathe light. That is, light, like air, is absorbed and in some way influences cellular function.

While one group of students of rickets has been groping for proof that the cause of the disease lay in physical conditions, another group has been too intent on the proofs of the curative properties of a dietary factor contained in cod liver oil for the proper consideration of other possibilities. Two factors, to all external appearances of a totally different nature, one

contained in cod liver oil and the other in light, are operative in the prevention and cure of rickets. This fact explains, in part at least, the confusion that has arisen concerning the etiology of the disease.

We do not regard the etiology of rickets as a solved problem. We do not doubt, however, that the demonstration of the curative action of light in rickets is a substantial step toward the complete understanding of the cause of this disease. Experiments with rats by Sherman and Pappenheimer[26] and ourselves[27] have shown that, in the absence of certain light rays and of a factor or factors contained in cod liver oil, a disease indistinguishable from the rickets of human beings may be produced through a deficiency of phosphates in the diet. Rickets so produced may be cured by cod liver oil or prevented by certain light rays. We have also shown that in rats fed on a diet in which the phosphate ion is sufficiently abundant, rickets does not develop even in the absence of certain light rays and of a factor or factors contained in cod liver oil.

Thus, experimentation with the rat makes it appear that deficiencies in light or in a factor or factors contained in cod liver oil do not cause rickets but rather permit defects in the inorganic composition of the diet to become effective. The disease is chiefly the expression in the skeleton of the disturbance of the inorganic metabolism in question. Our experiments have indicated that the function of light and of a factor or factors contained in cod liver oil, so far as rickets is concerned, is to exert a regulatory influence over the mineral metabolism of the body having to do with ossification and calcification. When the body is deprived of the regulatory action of certain light rays and of a factor or factors contained in cod liver oil, such disturbances in the inorganic metabolism of the body are permitted to develop.

As a result of these experiments with rats there is not the slightest doubt in our minds that either rickets or osteomalacia could be made to develop in human beings deprived of the protective influence of light rays and the dietary factor or factors contained in cod liver oil as easily as in the rat through similar alterations in the salt composition of the diet. Indeed, it may be that human beings are especially sensitive to even slight disturbances in the relations of the inorganic constituents of the diet when lacking the protective influence of light and the factor or factors contained in cod liver oil.

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[26] Sherman, H. C., and Pappenheimer, A. M.: Experimental Rickets in Rats, I, A Diet Producing Rickets in White Rats, and Its Prevention by the Addition of an Inorganic Salt, J. Exper. Med. **34**: 189 (Aug.) 1921.

[27] Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmonds, Nina: Studies on Experimental Rickets, III, A Pathological Condition Bearing Fundamental Resemblances to Rickets of the Human Being Resulting from Diets Low in Phosphorus and Fat-Soluble A: The Phosphate Ion in Its Prevention, Bull. Johns Hopkins Hosp. **32**: 160 (May) 1921.

Our experiments with the rat, therefore, make us think that in many instances the development of rickets in the human being may be due primarily to abnormal relations between certain mineral constituents of the diet; i. e., the disease has a purely exogenous origin. We are not at all certain, however, that the disease always has an exogenous origin. We think it probable, indeed, that rickets in some instances has an endogenous origin. It seems likely that in human beings, especially when deprived of the protective influence of certain light rays and of a factor or factors contained in cod liver oil, the mineral metabolism may be disorganized from causes operating within the body in such manner as to give rise to rickets.

SUMMARY

1. The object of the experiment was to determine whether or not sunlight prevents the development of rickets in the rat.

2. A diet was employed which at room light regularly gives rise to a disease in its essential features identical with rickets as seen in human beings. The diet was high in calcium, low in phosphorus and was insufficiently supplied with fat-soluble A. In other respects it was well constituted.

3. Eighteen rats were placed on the diet. Twelve were exposed to sunlight for a total of 242 hours over a period of sixty-two days. Six were kept under conditions of ordinary room light as control animals.

4. The control rats, killed with ether at the end of sixty days, all showed rickets.

5. The rats exposed to sunlight, killed coincidently, remained without exception entirely free from rickets. The absence of the lesions of rickets was confirmed by histologic examination.

6. The beneficial effects of the sun's rays were not limited to the skeleton, since the condition of the animals underwent a general improvement under the influence of the treatment with sunlight. The effect of the sunlight on the skeleton was a manifestation of its favorable effect only on a single tissue.

7. The exposure to the sun's rays, however, did not entirely compensate for the defects in the diet. The animals remained undersized; the bones, though completely calcified, remained thin. Though the sunlight did not alter the defects in the diet, it permitted the animals to thrive to a limited extent in the presence of them.

8. It is necessary to conclude, therefore, that the sunlight in some way raises the efficiency of the body cells. It enables the organism to put into operation regulatory mechanisms which otherwise would have been inoperative or ineffectual.

9. The effects of sunlight and of cod liver oil on the growth and calcification of the skeleton and on the animal as a whole seem to be similar, if not identical.

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STUDIES ON EXPERIMENTAL RICKETS

XV. THE EFFECT OF STARVATION ON THE HEALING OF RICKETS

By E. V. McCollum and Nina Simmonds

Department of Chemical Hygiene, School of Hygiene
and Public Health, The Johns Hopkins
University, Baltimore

AND

P. G. Shipley and E. A. Park

Department of Pediatrics, The Johns Hopkins
University, Baltimore

We have shown elsewhere[1] that certain types of faulty [31] diets induce bone changes identical with those seen in rickets in human beings. Cod-liver oil exerts a pronounced effect in preventing the development of these changes, or, once they are established, tends to cause the bones to return toward a normal condition.

The most effective way which we have found to demonstrate the therapeutic value of cod-liver oil is through the application of our " line test," which has been fully described in another paper.[2] Young rats are restricted to our diet (Lot 3143) which has the following composition for a period of 35-40 days.

Lot 3143

Wheat	33.0
Maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

Any other diet having identical dietary properties would serve the same purpose.

[31] This diet contains proteins of good quality and in great abundance (about 33 per cent), and about twice the calcium necessary for optimal growth and nutrition. It is, however, low in fat-soluble A and below the optimum in its content of phosphorus. One hundred grams contain 0.3019 gram of phosphorus.

If young rats are fed on this diet they develop a condition of the skeleton which is identical with the most severe rickets seen in the child. In the bones of these animals (Fig. 1) the cartilage is overgrown and abnormally persistent. The shaftward margin of the cartilage is irregular and the cartilage is invaded by blood vessels from the shaft of the bone. Lime salts are not deposited in the cartilage, so that no zone of provisional calcification is formed. A wide zone of tissue is formed between the cartilage and the shaft proper of the bone. This is made up of blood vessels, connective tissue and marrow elements, and of cartilage cells in all stages of degeneration, reversion, and of metaplasia into osteoid tissue. This is the rachitic metaphysis. The cortex of the shaft of the bone and the trabeculæ of the spongiosa are surrounded by wide zones of osteoid tissue (uncalcified bone). No signs of abnormal resorption of bone tissue are to be found. These bones are very soft and at autopsy the animals show deformities of the skeleton which we have discussed in detail in a preceding communication.[1]

When a young rat has been restricted for 35-40 days to this diet, experience has shown that the cartilage and adjacent portion of the metaphysis in the long bones will be entirely free from calcium salts. If now cod-liver oil is administered to the amount of 2 per cent of the diet, calcium salts are deposited in the cartilage on the epiphyseal side of the metaphysis. This amounts to the reappearance of the provisional zone of calcification. From the work of Schmorl and others we know this phenomenon to be the beginning of the healing of the lesion. The deposited salts appear in longitudinal sections [32] of the bone as a fine line crossing the proliferative zone of the epiphyseal cartilage transversely.

We have found that the administration of cod-liver oil to the amount of 2 per cent of the diet causes the line of calcification to appear in the cartilage within five days.

We have recently observed that when young rats with rickets are made to fast for periods of three to five days (distilled water only being offered), healing begins in exactly the

same way as it does when suitable amounts of cod-liver oil [32]
are administered.

Ten animals, as indicated in Table I, were fed on our diet No. 3143. At the expiration of the preparatory period they were placed in special cages and starved for 3-5 days.* They were then killed with chloroform and autopsied. The long bones were bisected longitudinally, immersed in a 1 per cent solution of silver nitrate, and examined under the microscope after exposure to light for the presence of a newly deposited provisional zone of calcification. The results of this gross examination were checked by the study of celloidin or frozen sections.

TABLE I

Data Descriptive of Rats Employed for Showing the Effects of Fasting on the Initiation of the Healing Process in Rickets

Number of rats	Age in days when put on diet	Days on diet	Age in days at death	Days starved	Sex	Weight at death
1182	60	70	130	5	♂	82
1221	45	64	109	5*	♂	87
1341	45	49	94	2*	♀	101
1345	55	49	104	3*	♀	85
1358	60	55	115	3*	♂	125
1364	65	30	95	3*	♂	80
1365	65	30	95	3*	♀	80
1366	65	30	95	3	♀	68
1368	60	35	95	3	♀	75
1369	60	35	95	3	♀	75

* Found dead on the morning of the day indicated.

The results of starvation were controlled by the examination of animals which had been raised in the same cages on

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* The cages employed for the fasting rats had wide mesh wire bottoms, so that the feces passed through, and it was impossible for the animals to eat their stools. No bedding of any kind was available for the rats to eat.

[32] diet No. 3143 at the same time, but had not been starved.[*] Not one of the starved animals failed to show re-formation of the provisional zone of calcification, and other evidences of the healing of the rachitic process. None of the controls had a vestige of calcium in the cartilage or metaphysis.

Since we have found that even on diet No. 3143 rats which were exposed daily to sunlight in the summer from the beginning of the experiment did not develop rickets,[3] three methods are now known of inducing the deposition of calcium salts in the cartilage of the bones of rachitic rats, viz., through starvation, exposure to light, and through the administration of cod-liver oil.

It is difficult to think of any single explanation for the common effect produced by cod-liver oil, sunlight and starvation on the skeleton of the rat rendered rachitic by means of a faulty diet. The possibility has been suggested that the effect of cod-liver oil may be attributable to physical force of one kind or another. This view we have discussed more fully in another place. But even if the effect of cod-liver oil should be the result of a physical force rather than a chemical reaction it does not follow that one could generalize regarding the other vitamins and accept the view that they necessarily act in an analogous manner. The analogy between the effects on the body of vitamin preparations and of the hormones is so striking that we are not justified in hastily abandoning the view that the vitamins are organic substances. Two at least of the hormones, thyroxin and adrenalin, are definite organic compounds.

Starvation causes healing of the rickets in a rat just as do cod-liver oil and sunlight, and the mechanism of the deposition of calcium salts in the proliferative cartilage may be the same. But cod-liver oil and sunlight improve the general condition of the animal. This is indicated by increased growth, longevity, muscular activity, storage of fat, and sexual potency. Starvation, on the other hand, causes the death of the rat in a few days. Its effects on the animal are favorable only in that

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[*] We have sections from the bones of nearly 100 animals which were maintained on diet No. 3143 under the same conditions as our test animals. These served as an additional control for the test.

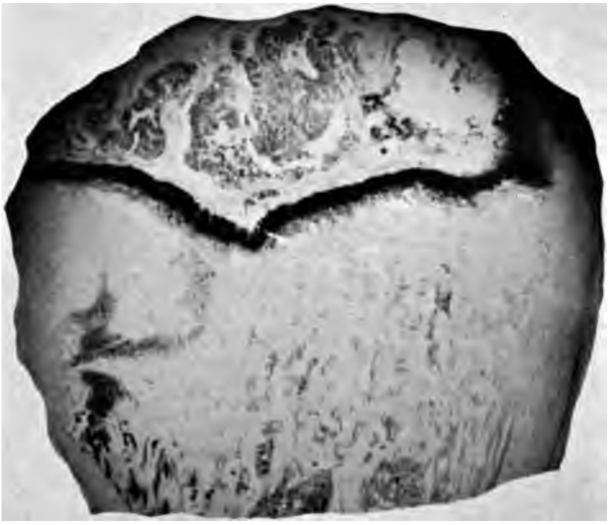

FIG. 1.—Photomicrograph of a section from the rachitic animal which had received diet No. 3143. There is no calcification of the epiphyseal cartilage or of the metaphysis.

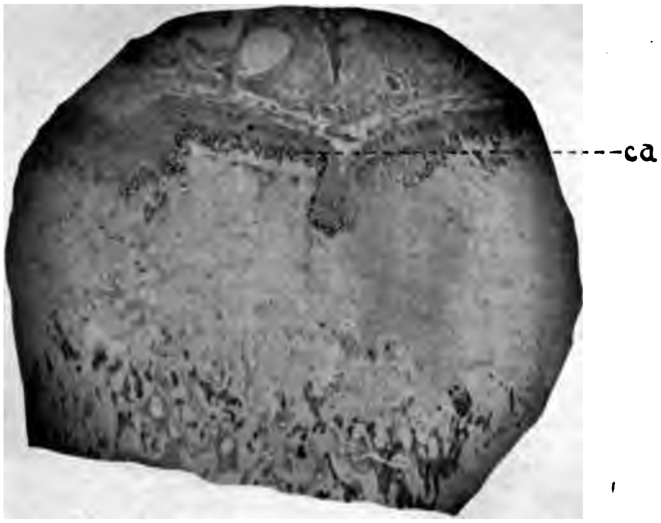

FIG. 2.—Photomicrograph showing the re-formation of a provisional zone of calcification (Ca) in the bone of a rachitic animal which had been starved for 5 days.

they cause the healing of rickets. The mode of action of the [32] three agents must be entirely different. It seems necessary to think that sunlight and cod-liver oil act by enabling the cells to carry on, even when their food supply is unfavorable. In other words, they aid the organism to adapt itself to an adverse environment. Starvation, on the other hand, must produce healing of the rickets at the expense of other body tissues. We cannot disprove at present the possibility that the cell functions more effectively when it is relieved of the load which a faulty diet imposes on it. However, during starvation certain tissues are destroyed for the upkeep of the supply of nutriment for those whose integrity must be safe-guarded to preserve life.

We have shown that the administration of cod-liver oil is followed by healing of rickets in rats even when they are receiving a diet with a faulty calcium-phosphate ratio. It may be that during the disintegration of protoplasm, which is consequent upon starvation, some substance is liberated which permits the animal to readjust the salt ratio within itself. But although it is possible that starvation liberates from the tissues an organic factor or factors, such as are in cod-liver oil, it is not necessary to account for healing in this way. Providing calcium and phosphorus are present in the proper proportions, normal calcification of the skeleton will occur. When the defects in the diet are such as they happen to be in the diet which we employed in these experiments (diet No. 3143), it would only be necessary for phosphorus to be liberated into the blood stream from disintegrated protoplasm (muscle for example) in order to cause calcium deposition to occur in the [33] cartilage. Animals on diet No. 3143, like rachitic children, have much less than the normal amount of phosphorus in the circulating blood. The administration of cod-liver oil or radiation from the mercury vapor quartz lamp causes the phosphorus in the blood to rise and it is not improbable that the healing of rickets which results from starvation is the immediate result of an equivalent increase of this element in the circulating blood. Just as soon as the load of a defective diet is removed and the body is forced to draw on its own tissues for maintenance of life and function, stored foodstuffs are re-

[33] leased into the blood stream as the result of a process of selective tissue decomposition.

These experiments furnish the first anatomical proof of the beneficial effect of starvation on the animal body. The good effects of fasting are given a new meaning because the organism is able to adapt itself to pathogenic distortions of normal metabolic ratios when the burden of carrying on exogenous metabolism is removed. Since the starving body is capable of readjusting abnormal relations within itself it is easy to understand the benefit derived by a diabetic from occasional hunger days, and why it is that the wasted athreptic infant does not develop rickets.

Rickets has certain of the characteristics of a deficiency disease because a certain substance contained in cod-liver oil and elsewhere corrects an anatomical condition which develops when the calcium and phosphorus in the diet are present in wrong proportions. Yet rickets has a feature entirely distinct from beri-beri, scurvy, and xerophthalmia. The relation between two inorganic elements determines the extent of the animal's need for the organic factor which cod-liver oil furnishes. No such relationship between a vitamin and any other food substance has been clearly demonstrated in any other condition.

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STUDIES ON EXPERIMENTAL RICKETS.

XVI. A DELICATE BIOLOGICAL TEST FOR CALCIUM-DEPOSITING SUBSTANCES.

BY E. V. McCOLLUM AND NINA SIMMONDS,

(From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

AND P. G. SHIPLEY AND E. A. PARK.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

PLATES 1 AND 2.

(Received for publication, January 11, 1922.)

In our second communication (1) we described two diets (Lots 2638 and 2677) which we had used to prepare animals for a biological test for the calcium-depositing power of various food substances. This test depends on the power of a given substance to cause the reappearance of a provisional zone of calcification in epiphyseal cartilages of animals with very severe rickets, whose cartilages had been rendered calcium-free by faulty diets. We called this procedure the "line test," because in gross specimens or in histological preparations the new zone of calcification appears as a line of calcified tissue crossing an area which is free from lime salt deposits. Schmorl (2) showed that the first sign of healing in rachitic children was the reformation of the provisional zone of calcification on the epiphyseal side of the metaphysis. The value of the reaction is dependent upon the certainty with which a given diet will operate to produce bones in which the epiphyseal cartilage and the metaphysis are calcium-free. In other words, it is not sufficient that the diet shall produce rickets. It must cause an exaggerated form of florid rickets to develop without fail. Our results with the above diets were not entirely satisfactory, since now and again an animal would show some evidence of calcification of the epiphyseal cartilage. Furthermore, we found that young rats on these diets did not grow enough to produce as severe rickets as we desired. Many animals died in a short time because of the pronounced deficiencies of the diet (Lot 2677). Moreover, rats on these diets showed a marked tendency to destroy and eat their companions in the cage. This, of course, vitiated the experiments.

The data presented in the earlier papers of this series leave no room for doubt that certain faulty relations between three dietary factors, *viz.* calcium, phosphate, and an unidentified organic substance, lead to the development of the picture of rickets in young rats as it occurs in children. When a diet contains a somewhat excessive amount of calcium, decidedly less than the optimal amount of phosphate, and very little of an organic substance which is especially abundant in cod liver oil, rats which are fed on it develop rickets (3). We have pointed out that pronounced rickets develops only when the conditions are such as to permit young rats to grow. When the faults in the diet are such as to cause rickets, the severity of the disease is increased if the food is such as to insure to the animal a certain amount of growth and to keep up its vitality.

A diet which proves highly satisfactory for the purpose in hand, in that young rats fed upon it have marked rickets with an epiphyseal cartilage and metaphysis uniformily free from calcium, is made up as follows:

Diet 3143.

Whole wheat kernel	33.0
" maize "	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

100 gm. of this diet contains 1.221 gm. of calcium, and 0.3019 gm. of phosphorus.
Weight ratio Ca:P = 4.04:1

Atomic ratio Ca:P = 1:0.319

The proteins of this diet are of good quality and are abundant (about 33 per cent of the food mixture). The amount of phosphorus in the mixture is distinctly below the need of the growing rat for this element. 100 gm. of this food contain 0.3019 gm. of phosphorus. Our observations indicate that the optimal amount of phosphorus in the diet of the growing rat is not less than 0.4146 per cent. It may be considerably higher. The calcium content of the food mixture is 1.221 gm.—approximately double that required for optimal growth and function. The content of fat-soluble A is very low but suffices to prevent the development of xerophthalmia, and to make growth possible for a time, other factors permitting. The content of the second organic factor supplied by certain fats (if this proves to be a distinct entity) is likewise very low.

Young rats which have grown rapidly to about 55 to 60 gm. (35 to 40 days old) are confined to Diet 3143 for a period of about 35 to 40 days (preparatory period). If the animals are allowed to remain too long on this diet they deteriorate physically and their hind legs become paralyzed. After being confined to the rickets-producing diet for from 35 to 40 days the rats have some difficulty in using their hind legs. The changes in the animals' gait are not so striking that they would be noticed except by one who is familiar with the habits and movements of the rat. The gait is tottering, and the hind quarters waver slightly from side to side. When the animal starts to move off rapidly it hops, usually favoring one hind leg. We have never failed to find the cartilages of the bones of animals in this condition calcium-free. The metaphysis is usually quite free from calcium salts but occasionally a few calcified areas are found.

When a group of rats are ready to be used for the test, some of them are given the substance which we desire to study, in their food, and the rest serve as control animals and continue to receive Diet 3143 unchanged. (The time during which the rats receive the substance which is being studied may be called the "test period.") The test animals and controls are killed and autopsied at the end of a designated time. It has been our custom to examine the distal end of the left femur and the proximal end of the left tibia for reformation of the provisional zone of calcification. Of the two bones the tibia is perhaps the better, since the epiphyseal line is straighter in this bone than in the femur, and the metaphysis wider.

The bones are split in two with a sharp scalpel and one-half of each is fixed in 10 per cent formaldehyde, decalcified in Müller's fluid, and embedded in parloidion. The other half of each bone is immersed in a 1 per cent solution of silver nitrate and exposed to sunlight or to the light of a Mazda lamp, and studied through a binocular microscope. The slices which have been treat silver nitrate may be cut into frozen sections or may be p as permanent gross specimens. For the latter purpose t washed well with water, decolorized with a solution of thiosulfite, rewashed and kept in formaldehyde.

The bones of these animals are very soft. The ends a much enlarged. On section the cartilage of the epiph irregular in depth and the epiphysis is separated from t physis by a zone of white tissue (the metaphysis) which about 0.5 cm. deep. In the bones of the control animals which give a negative "line test" there is no calcificatio epiphyseal cartilage, and little if any in the metaphysis The shaft is incompletely calcified. We have describe animals and their bones completely in another place (3) which give a positive "line test" differ from the controls in a broad, linear deposit of calcium salts on the metaphys of the epiphyseal cartilage. The band, which may not be plete, is separated from the shaft of the bone by the the metaphysis, and from the nucleus of ossification of the physis by the depth of the epiphyseal cartilage. It can be on the freshly cut surface of an untreated bone as a yellow which marks the epiphyseal border of the metaphysis. deposit is blackened by silver nitrate in fresh or fixed g specimens. It appears like a cross-section of a black hon comb when it is examined with a binocular microscope. T metaphyses of these bones usually appear to be congested. T deposit is in the proliferative zone of the cartilage. It m extend completely across the bone or may be interrupted or fr mentary according to the activity of the calcium-depositi substance (Fig. 2). It is stained brown by silver nitrate and a intense blue by hematoxylin. It is separated from the calcified trabeculæ of the shaft by the width of the metaphysis. The honeycomb-like appearance of the line is explained by sections which show that the matrix of the cartilage only is calcified (Fig. 3). The cartilage cell is not. The deposit is granular.[1] Although the possibility of detecting this calcification in living n by the use of the x-ray has been recognized for sometime Park and Howland (5) were the first to make use of it experi- y. They were able to show that cod liver oil would ly induce calcification and healing in the bones of children ets. Since their experiments Huldschinsky (6) and others demonstrated the curative power of ultra-violet light in the way. When rats are used as subjects the picture on the plate may be confirmed by microscopic study of the bones e results obtained with this test are illustrated by the effect administration of cod liver oil. Within 5 days the addition cent of cod liver oil to the food causes complete recalci- of the cartilage.

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[1] In studying fresh, silvered preparations it must be borne in mind that a precipitate of silver is sometimes thrown down irregularly on the surface of the bone without relation to calcium deposits. If this precipitate is

iver oil added to the food in amounts equal to only 0.2 or ent of the food mixture does not cause the line of calcium ate to appear in 5 days. 0.6 per cent, on the other hand, the appearance of an irregular calcification in some of bones in this time. We shall describe later the effects of ll doses of cod liver oil repeated for shorter or longer periods roducing the reformation of the provisional calcified zone.

n conducting these tests it is necessary to keep accurate records the food consumption by both test and control rats during the erval following the administration of the substance to be ted. This is so because starvation causes rats with rickets to posit calcium salts in the epiphyseal cartilage, just as does cod ver oil (7). We have taken this precaution in all our tests. A sitive test can be relied upon only when the animals eat enough.

Since the amount of and the relation between calcium and phosphorus in the food is a very important factor in determining the ability of the skeleton to grow normally, it is essential, when employing the test here described for the examination of a natural food (*e.g.* the leaves of plants, etc.), to modify the basal diet at

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formed on the cartilage it may be mistaken for an irregular calcification. The regular honeycomb appearance of the true "line" of calcification distinguishes it. In case of doubt the bone may be treated with sodium thiosulfite. The blackness of the silvered calcium is unaffected by 2 to 3 minutes' treatment with the solution, but the precipitate will be dissolved at once. We have always controlled the results of the examination of fresh tissues by the study of microscopic sections. The results obtained by the use of the two methods have never failed to correspond.

TABLE I.

Rat No.	Age when put on diet.	Days on diet.	Weight at death.	Sex.	Control.	Days received diet plus cod liver oil.	Food eaten in 5 days.	Healing.
	days		*gm.*				*gm.*	
1178	60	70	73	♀		1.0 per cent cod liver oil for 5 days.	1*	+
1179	60	70	108	♀		0.6 " " " " " " 5 "	28	Irregular deposit.
1180	60	70	98	♀		0.4 " " " " " " 5 "	29	0
1181	60	70	117	♀		0.2 " " " " " " 5 "	29	0
1220	45	64	92	♂		1.0 " " " " " " 5 "	22	+
1348	45	49	107	♂		2.0 " " " " " " 5 "	36	+
1349	45	49		♂		2.0 " " " " " " 5 "	33	+
1350	45	49	77	♀		2.0 " " " " " " 5 "	29	+
1352	55	49	90	♀		2.0 " " " " " " 5 "	40	+
1323	40	39	75	♂	Control.		35	0
1334	50	57	73	♀	"		37	0
1335	50	57	105	♀	"		35	0
1336	50	57	85	♀	"		38	0
1224	55	70	130	♀	"		39	0
1225	35	70	86	♀	"		36	0
1354	45	54	100	♀	"		37	0
1337	50	47	110	♂	"		37	0
1355	45	54	135	♂	"		39	0

* Comparable to starvation. **Rat was paralyzed in hind legs and was in helpless condition.**

the time of making the test, so that the added food will make no change in the inorganic composition of the diet as compared with that of the food in the preparatory period. In another paper in this series we show in some detail how important it is to adhere rigidly to the composition laid down for the diet used for the preparation of the animals for this test.

The test is applicable for the demonstration of the effect of light rays or other radiations, or other remedial agencies. It constitutes, therefore, a valuable aid to further experimental work in the field of bone pathology.

SUMMARY.

1. A diet is described which is low in phosphate and in an undetermined organic substance and relatively high in calcium. This diet uniformly causes the bones of animals fed on it to be free from calcium as regards the epiphyseal cartilages and the metaphyses. These bones show an exaggerated type of florid rickets.

2. The reappearance of the provisional zone of calcification after the addition of any substance to the rickets-producing diet (beginning healing of the rickets) constitutes a test for the calcium-depositing power of the substance (the so called "line test").

3. Cod liver oil to the extent of 2 per cent by weight of the diet causes the provisional zone of calcification to be reformed in these bones in 5 days (a positive "line test").

4. 0.4 per cent of cod liver oil fails to cause the provisional zone of calcification to reappear in the bones. 1 per cent of cod liver oil for 5 days does not cause so complete a calcium salt deposition as does 2 per cent.

5. Both test and control animals must be kept under the same conditions of illumination, and since starvation causes redeposition of calcium in the bones, records of the food consumption of the animals must be kept during the experiment.

6. The basal diet must be so modified when any natural foods are tested that the addition of the food to be examined will not change the inorganic content of the mixture used in making the test from that of the original diet used during the preparatory period.

7. This test may be used to determine the ability of any agency to heal rickets.

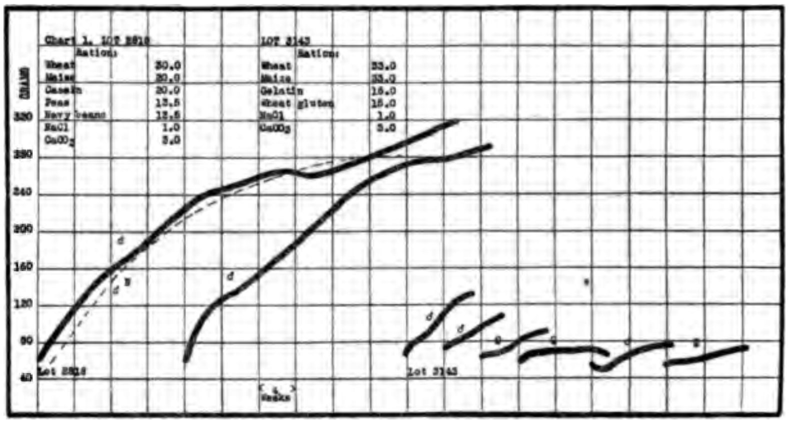

Chart 1. The curves of Lots 2818 and 3143 illustrate the importance of having an optimal ratio between calcium and phosphorus in the diet of the growing rat.

Lot 3143 had a diet which contained an abundance of proteins (33 per cent) of good quality, but was too low in phosphorus and too high in calcium. The diet contained 1.221 gm. of calcium in 100 gm. This is about twice the optimal amount of this element. 100 gm. contained 0.302 gm. of phosphorus. This we know to be distinctly below the optimal amount, although the most favorable concentration of this element in the diet has not been accurately determined. In certain other diets we have been able to secure good nutrition when the food contained 0.415 per cent of phosphorus. We may safely assume that the diet under consideration is at least 30 per cent too low in this element. In addition to these defects the diet of Lots 3143 and 2818 was relatively deficient in fat-soluble A.

The diet of Lot 2818 differed essentially from that of Lot 3143 in its content of phosphorus. As respects protein, calcium, and fat-soluble A, they were essentially alike. Diet 2818 contained 0.4928 per cent of phosphorus, and 1.247 per cent of calcium. The calcium: phosphorus ratio in each of these rations were:

Lot 3143.	*Lot 2818.*
Atomic ratio ... Ca:P::1:0.319	Atomic ratio ... Ca:P::1:0.523
Weight ratio Ca:P::4.04:1	Weight ratio Ca:P::2.51:1

The diet of Lot 3143 contained only 61.25 per cent as much phosphorus as did the diet of Lot 2818. This difference in phosphorus content was sufficiently significant for the welfare of the growing rat to make the differences in growth seen in the contrasting curves of the two groups shown in the chart.

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EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. This figure shows the distal end of a section of the tibia of a control animal which had been fed our diet, No. 3143. Note the proliferative zone of cartilage (*C*) which is entirely free from calcium salts, and the wide calcium-free zone (*M*) between the cartilage and the end of the shaft (*D*) which contains partially calcified trabeculæ.

FIG. 2. This figure shows the distal end of the tibia of an animal which had given a positive line test. This animal had been fed diet, No. 3143, but had received 2 per cent of cod liver oil for 5 days before death. Note the "line" (*CA*) of newly deposited calcium in the proliferative cartilage. This line is a newly formed zone of provisional calcification.

PLATE 2.

FIG. 3. Higher magnification, × 300, of the newly formed provisional calcified zone (*CA*). This picture shows the calcification of the intercellular matrix of the proliferative cartilage. Some of the calcified cell capsules have been broken open and invaded by capillary vessels from the shaft.

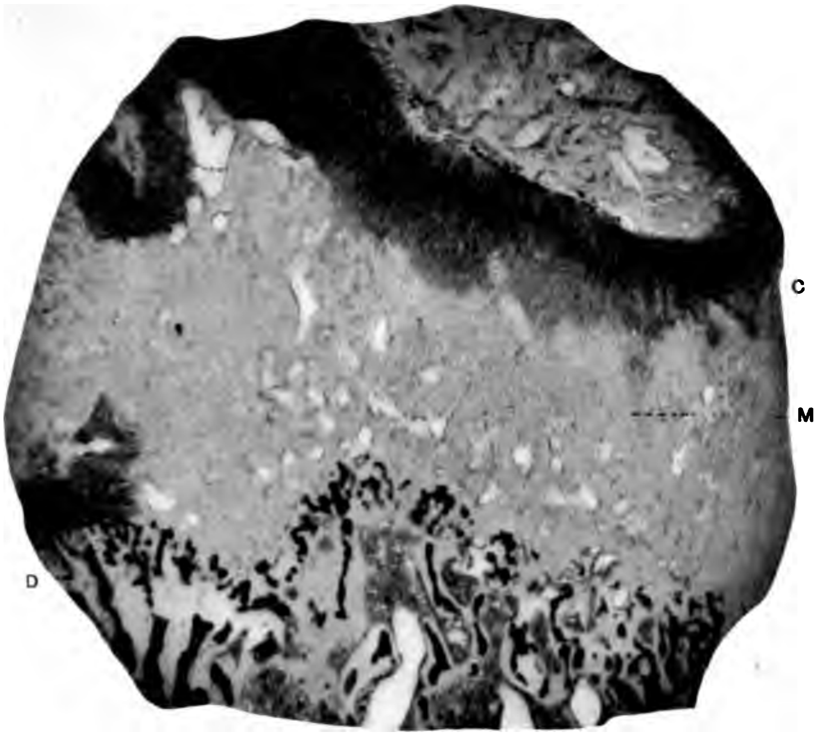

FIG. 1.

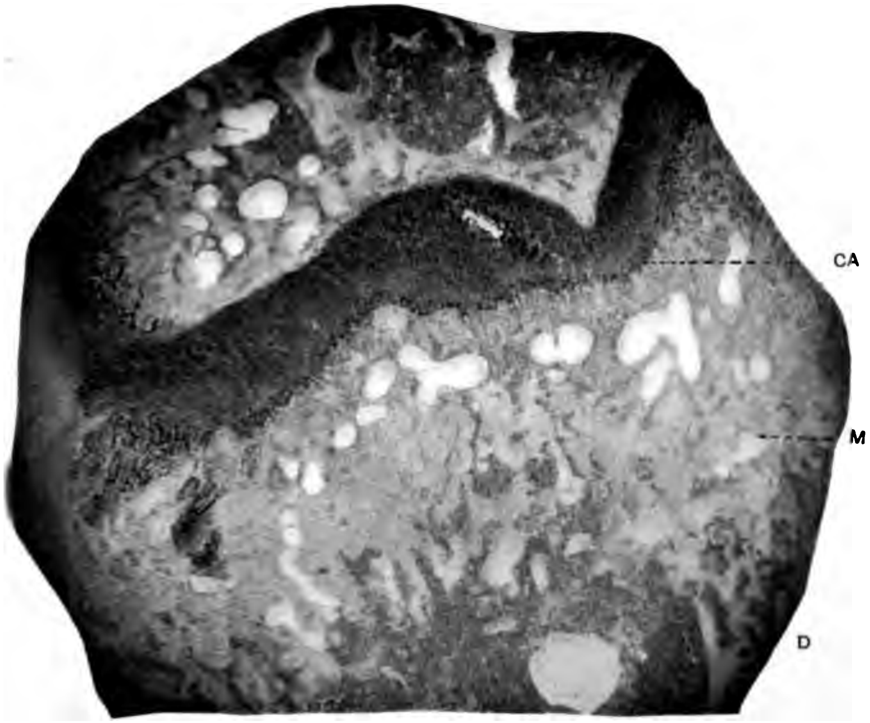

FIG. 2.

(McCollum, Simmonds, Shipley, and Park: Studies on experimental rickets. XVI.)

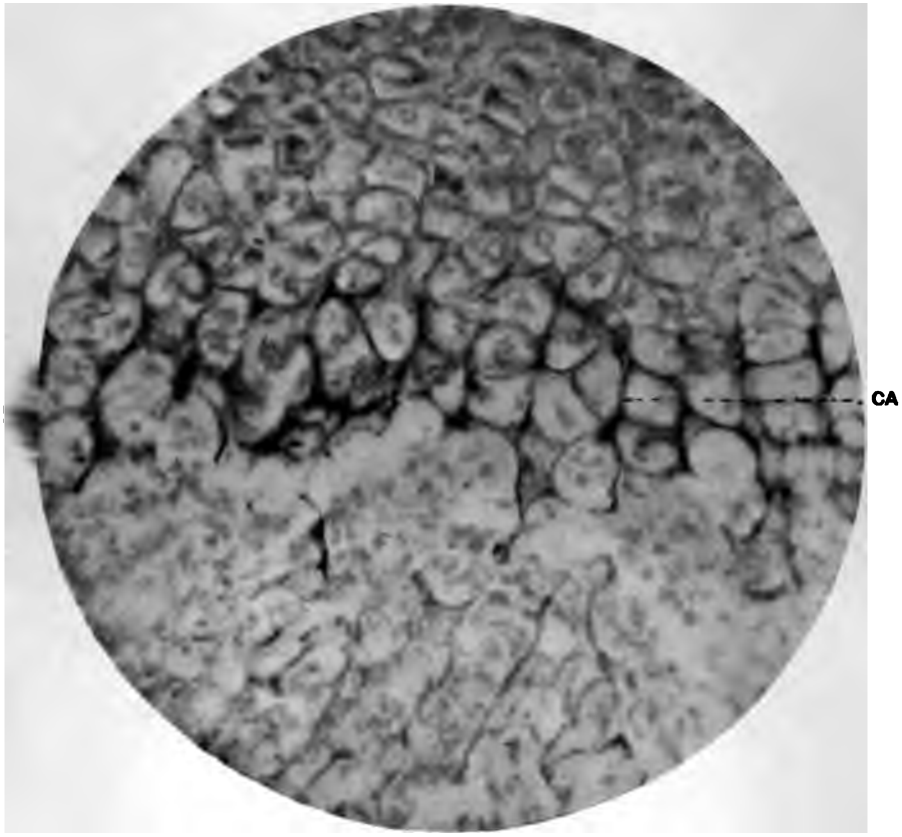

FIG. 3.

(McCollum, Simmonds, Shipley, and Park: Studies on experimental rickets. XVI.)

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STUDIES ON EXPERIMENTAL RICKETS. XVII.

The Effects of Diets Deficient in Calcium and in Fat-soluble A in Modifying the Histological Structure of the Bones.

By E. V. McCOLLUM, NINA SIMMONDS, and MAY KINNEY,

Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore,

AND

P. G. SHIPLEY and E. A. PARK,

Department of Pediatrics, Johns Hopkins University, Baltimore.

(Received for publication, November 20, 1921.)

Plates I to VII.

In a previous paper (1) we described experiments on the growing rat which were undertaken to determine the effects of diets extremely low in calcium, but which were good in other respects. The fat-soluble A* was furnished in the form of butter fat. The diets had the following composition:

Diet 2684.	
Wheat	30.0
Maize	20.0
Rice (polished)	12.0
Rolled oats	11.5
Peas	11.0
Navy beans	11.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	2.0

Diet 2810.	
Wheat	30.0
Maize	14.5
Rice (polished)	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Beefsteak (round)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

Ration 2684 consists of entire cereal grains and legume seeds with the exception of polished rice, supplemented with common salt, sodium

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* Anti-xerophthalmic substance.

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STUDIES ON EXPERIMENTAL RICKETS. XVII.

THE EFFECTS OF DIETS DEFICIENT IN CALCIUM AND IN FAT-SOLUBLE A IN MODIFYING THE HISTOLOGICAL STRUCTURE OF THE BONES.

By E. V. McCOLLUM, NINA SIMMONDS, and MAY KINNEY,

Department of Chemical Hygiene, School of Hygiene and Public Health, Johns Hopkins University, Baltimore,

and

P. G. SHIPLEY and E. A. PARK,

Department of Pediatrics, Johns Hopkins University, Baltimore.

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Plates I to VII.

In a previous paper (1) we described experiments on the growing rat which were undertaken to determine the effects of diets extremely low in calcium, but which were good in other respects. The fat-soluble A* was furnished in the form of butter fat. The diets had the following composition:

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Butter fat	2.0

Diet 2810.

Wheat	30.0
Maize	14.5
Rice (polished)	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Beefsteak (round)	10.0
NaCl	1.0
Dextrin	1.5
Butter fat	5.0

Ration 2684 consists of entire cereal grains and legume seeds with the exception of polished rice, supplemented with common salt, sodium bicarbonate sufficient to make a slight excess of alkaline over acid radicals, and 2 per cent. of butter fat added to supplement the diet with respect to fat-soluble A. The amount of butter fat added was not enough to bring the content of fat-soluble A up to the optimum.

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* Anti-xerophthalmic substance.

100 grams of this food mixture contained 0.0323 gram of calcium and 0.3631 gram of phosphorus.

Ration 2810 differed from ration 2684 in that it contained 10 per cent. of beefsteak, more butter fat (fat-soluble A) and no sodium bicarbonate. Its proteins were of excellent quality. Its content of fat-soluble A was approximately the optimal. It was somewhat richer in phosphorus than ration 2684. The calcium content of both diets was far below the optimal. Our experimental work with the rat indicates that a content of 0.641 gram of calcium per 100 grams of ration is very close to the optimum concentration of this element.

Ration 2810 contained in 100 grams 0.0498 gram of calcium (Ca) and 0.3900 gram of phosphorus (P). It contained only 7.7 per cent. as much as our studies indicate to be the optimal content of calcium. When 1.5 per cent. of calcium carbonate is added to this diet it becomes very satisfactory, as is shown by the fact that we have seen five successive generations of rats successfully nourished upon it.

These diets produced histological changes in the bones which have certain fundamental resemblances to, but also present certain differences from, the lesions characterizing rickets in the human being.

In the present paper we wish to describe experiments undertaken to determine the effects on the bones of a diet obtained from cereals and legume seeds unsupplemented (ration 2638); also the same diet supplemented with sodium chloride and sodium bicarbonate (Lot 2249). Our observations indicate that neither of the two salts just mentioned exert any direct influence on the structure of the bones.

Diet 2638.	
Wheat	30.0
Maize	30.0
Polished rice	10.0
Rolled oats	10.0
Peas	10.0
Navy beans	10.0

Diet 2249.	
Wheat	30.0
Maize	27.5
Polished rice	10.0
Rolled oats	10.0
Peas	10.0
Navy beans	10.0
NaCl	1.0
$NaHCO_3$	1.5

The diet of Lot 2638 was deficient in calcium, fat-soluble A, and a second dietary essential associated with certain fats, notably cod liver oil, provided this latter factor is a distinct entity; also, in a minor degree in sodium, chlorine and phosphorus. One hundred grams of the diet contained 0.0526 gram of calcium and 0.3646 gram of phosphorus.

The diet of Lot 2249 was deficient in the same factors as that of Lot 2638, except that it contained added sodium chloride and bi-carbonate. It contained less phosphorus than the optimum, but this deficiency was not so marked but that specially planned experiments are necessary to demonstrate that the well-being of rats can be improved by including in their diet a higher content of this element than is furnished by any ordinary seed mixture.

Diet 2638 produces histological abnormalities in the bones. These will also be considered in relation to the histological structure of the bones of animals in Lots 2810 and 2684. The latter two diets contained sufficient fat-soluble A (in butter fat) to maintain good growth and fertility, when other dietary factors are satisfactory.

Thirty-two rats were fed upon ration 2638. Some of them may be said to have received the faulty diet as soon as they were born, inasmuch as the mother animals were placed upon the ration prior to the birth of their litters, and were kept upon it throughout the period of lactation. We have shown, however, that the milk produced by a lactating rat confined to a faulty diet is a better food for her young than her diet is, as a source of nutriment for her. Up to about the age of 20 days, therefore, these young had the advantage of their lactating mothers as a factor of safety in their nutrition. Others were weaned to the faulty diet, i.e., they began to take the deficient ration as soon as they were able to eat solid food, viz.: on the 19th to 21st days. The other animals were not given the faulty ration until the 35th, 40th, or in the case of one rat, the 50th day. The rats were kept on the faulty ration for variable periods, ranging between 38 and 104 days, and averaging about 71 days.

THE EFFECTS OF RATION 2638.

The animals confined to the faulty ration, 2638, grew poorly or not at all. The animals started on the faulty ration at birth (through the mother), or soon after, reached at the end of 2 months a weight, varying in a litter of four, between 25 and 37 grams. (The average weight of a rat 20 days old is 35 grams, and the average weight of a rat 2 months old is 125 grams.) The rats placed upon the ration, after the basophilic cells found in the neighborhood of the trabeculae. Many evidences of actively progressing resorption could be found in both trabeculae and cortex.

The rats most affected by the faulty ration displayed deformities of the thorax and extremities so marked as to be visible in the living animal. The thorax was greatly flattened from side to side, and the lower part of the sternum, in consequence, was pushed forward with the production of the pigeon breast deformity. The cartilages and ribs met at sharp angles which pointed into the interior of the thorax. The costo-chondral junctions did not appear to be greatly enlarged, although greatly deformed. The posterior ends of the ribs were enlarged. The spinal column was bent. The ends of the long bones, for example, the ends of the radius and ulna at the wrist, and the femur and tibia at the knee, were enlarged. The shafts were slender, fragile, and soft. On examination of the cut surface of the femur or tibia, after longitudinal section, the epiphyseal cartilage could be seen to be of irregular depth, and to be separated from the shaft proper by a zone of white tissue. The cortex in some animals was very thin, but in others appeared thick and spongy. In these the marrow cavity was narrowed.

Before contact was established with the metaphysis all traces of columnar arrangement of the proliferative zone might or might not be lost, and its cells frequently showed no traces of any orderly arrangement. At the same time the cells themselves and their capsules expanded and became globular. The nucleus was vesicular with a fine chromatin network. The cytoplasm underwent a progressive loss of its affinity for basic dyes and the capsule became less distinct. In this condition many of the cartilage cells were incorporated in the trabeculae which formed the metaphysis. In some instances they retained their individuality, in other instances they became indistinguishable from osteoid corpuscles. Many underwent a progressive reversion to a form like that of thin fusiform connective tissue cells. Many rapidly degenerated in the trabeculae. They became flattened in the direction of the long axis of the bone. The fusiform nuclei stained less deeply with haematoxylin. Nucleus and cytoplasm became less and less distinct, and finally the cell disappeared, leaving a space in the osteoid which was only to be seen by its refractive edges. Usually the cartilage cell lost the characteristics of the parent tissue almost at once after contact with the metaphyseal vessels was established, and there was only a moderate tendency either to overgrowth or to persistence of the cartilage. In other words the change of the cartilage cell into an osteoid corpuscle was very abrupt in relation to the rate of growth of the metaphysis. Long tongues of unchanged cartilage, however, persisted, extending from the epiphyseal line into the depths of the metaphysis. Unchanged cartilage cells, singly or in groups, could be identified, scattered about in the metaphyseal osteoid, especially in the periphery of the bone. Cartilage cells which retained their staining characteristics in the metaphysis were apparently those which had been protected in some way from the influence of the blood vessels with which this region is filled. The cells in the tongues of cartilage which projected below the epiphyseal line were liable to a reversion to the morphology and the staining characteristics which were shown by cells in the proliferative zone of cartilage. They were heavily stained by basic dyes. The nucleus frequently became pyknotic, and the cells were flattened, as though they were under pressure in the direction of the long axis of the bone. They had a tendency to be shaped like thin wedges or crescents, or to have a narrow elongated fusiform shape. Persistence of these tongues of cartilage was most apt to occur at the periphery of the bone. The cartilage in these animals was much more liable to disruption from invasion by blood vessels than any other that we have examined. Invasion was usually carried on by single capillary loops which forced a few cells out of their places, but in many animals large areas of cartilage were destroyed before the inroads of large tufts of small vessels which sprouted from the diaphyseal trunks. The cartilage contained no deposits of lime salts.

The metaphyses of these bones were in a large part apparently the product of the diaphysis. Besides the tongues of cartilage described above, the metaphyses contained cartilage cells isolated or in groups. These might be unchanged or in any state of transition into osteoid corpuscles or degeneration. The trabeculae of osteoid tissue which made up the greater part of the metaphysis were the result largely of the activity of osteoblasts. They were separated, one from the other, by spaces which contained the blood vessels and connective tissue. The connective tissue might be dense, or delicate, like granulation tissue. It surrounded the blood vessels and represented the replacement fibrosis of the marrow which is frequently seen in the rickets of children, and has been held to have a causal relation to disease. Fibrosis of the spaces between the trabeculae in the metaphysis was the basophilic cells found in the neighborhood of the trabeculae. Many evidences of actively progressing resorption could be found in both trabeculae and cortex.

The rats most affected by the faulty ration displayed deformities of the thorax and extremities so marked as to be visible in the living animal. The thorax was greatly flattened from side to side, and the lower part of the sternum, in consequence, was pushed forward with the production of the pigeon breast deformity. The cartilages and ribs met at sharp angles which pointed into the interior of the thorax. The costo-chondral junctions did not appear to be greatly enlarged, although greatly deformed. The posterior ends of the ribs were enlarged. The spinal column was bent. The ends of the long bones, for example, the ends of the radius and ulna at the wrist, and the femur and tibia at the knee, were enlarged. The shafts were slender, fragile, and soft. On examination of the cut surface of the femur or tibia, after longitudinal section, the epiphyseal cartilage could be seen to be of irregular depth, and to be separated from the shaft proper by a zone of white tissue. The cortex in some animals was very thin, but in others appeared thick and spongy. In these the marrow cavity was narrowed.

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The pathological condition in the most severe cases caused by faulty ration 2638, however, differed in certain details from that usually found in the human subject of rickets:

1. The metaphysis had a more orderly arrangement. It was composed chiefly of broad trabeculae which, though entirely uncalcified, were disposed in a more or less regular manner. In the child with advanced rickets the metaphysis is a mixture of cartilaginous and diaphyseal elements intermingled in the utmost confusion.

2. The cartilage seemed to undergo a more sudden transformation into osteoid than in the human subject. In the rachitic child the process of transformation of cartilage into osteoid, and the degeneration of the cartilage seems to go on slowly. There were found, however, scattered through the metaphysis cartilage cells in all stages of degeneration or reversion to early states of development, or metaplasia into osseous or connective tissue elements.

3. The evidences of resorptive activity were especially marked.

4. Cells filled with basophilic granules lay scattered about in the immediate vicinity of the trabeculae.

In the last two respects the bones of the rats on diet 2638 were like those found in animals after feeding diets 2684 and 2810. These last mentioned diets were characterized also by a low calcium content and an amount of phosphorus slightly above the lower limit of the optimal for growth and maintenance in the rat. The animals on diets 2684 and 2810 were, however, supplied with a much larger amount of the fat-soluble A in the form of butter fat—2–5 per cent. of the total ration. The cartilage of certain rats on diet 2638, however, was disorganized to a much greater extent than those of animals on 2684 and 2810.

The condition commonly found in the bones in human rickets was more closely imitated in the bones of rats maintained on our diets 3127, 3143, and 3133, which were low in their content of phosphorus and insufficiently supplied with the fat-soluble A, but which contained optimal or slightly excessive amounts of calcium salts (3). Comparison of the bones of animals, fed diet 3133, with those fed diet 2638, shows that in the reaction excited by feeding the former type of diet the growth and persistence of cartilage was much more exaggerated. Diets of the type of 2638 induce in the most severe cases a pathological change, in the course of which destruction, both of bone and cartilage is much more pronounced. Certainly, however, as is demonstrated by the illustrations, the pathological condition produced by ration 2638 can be said to have borne fundamental resemblances to rickets as that disease ordinarily manifests itself in the bones of the child, and must be considered as closely related to the rickets of the human being.

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3. Pommer.
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EXPLANATION OF PLATES.

Plates I to V.

Figs. 1, 2, 3, 4 and 5. These photomicrographs were taken of sections from the distal ends of the femura of rats which had been given the diet of lot 2638. These bones show every gradation between severe rickets (Fig. 1) and a condition in which osteoporosis is almost completely developed (Fig. 5), with intense resorption and complete calcification of the proliferative cartilage.

Plate VI.

Fig. 6. Higher magnification than the preceding figures, to show the persistence of the cartilage cells, their degeneration and the invasion of the cartilage by blood vessels. Leitz achromatic objective No. 3. No ocular.

Fig. 7. High power photomicrograph showing osteoid tissue about the trabeculae of bone. Leitz achromatic objective No. 6. No ocular.

Plate VII.

Fig. 8. This picture shows in detail the "degeneration" of the cartilage cells and their direct transformation into osteoid corpuscles. Leitz achromatic objective No. 6. No ocular.

Fig. 9. Large cells with basophilic granulations (connective tissue mast cells clustered about trabeculae which show marked signs of resorption. Leitz achromatic objective No. 6. No ocular.

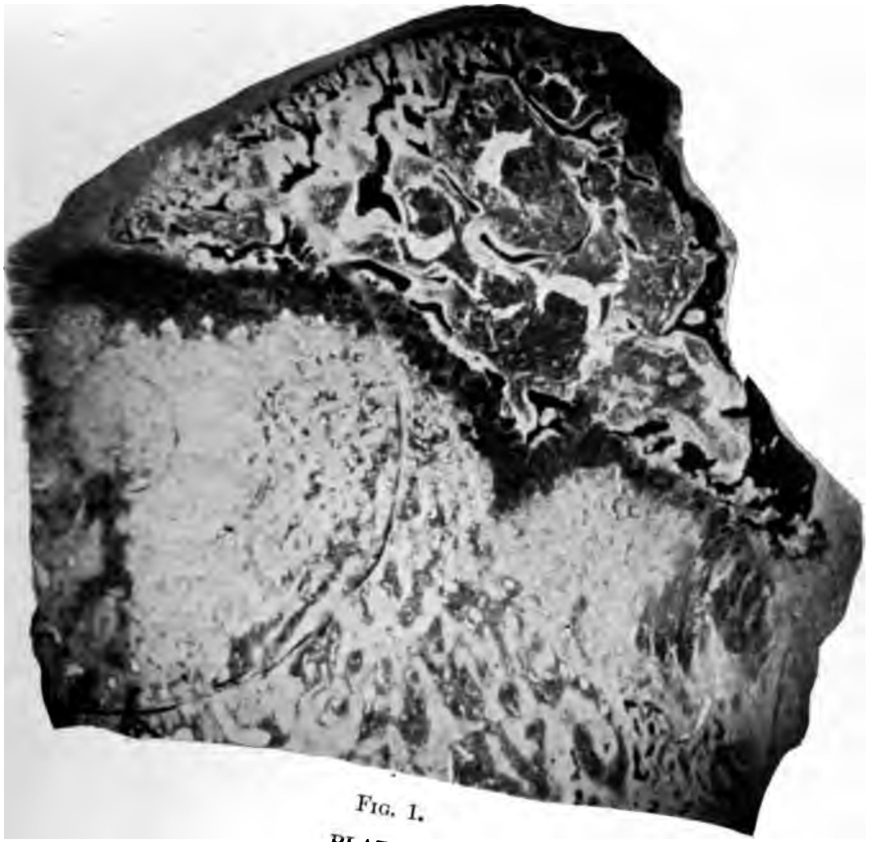

Fig. 1.

PLATE I.

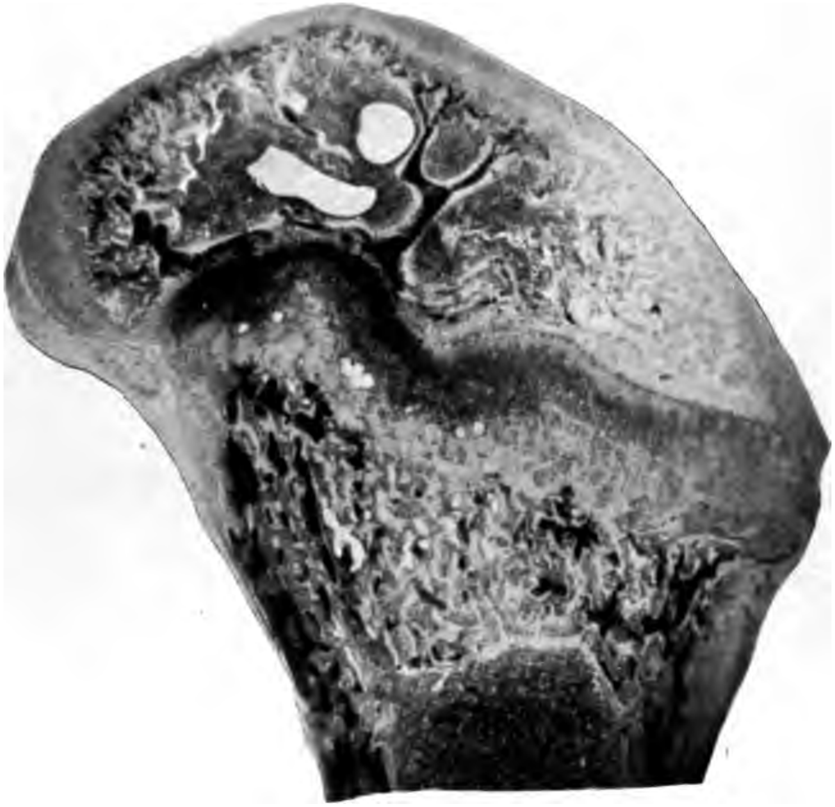

Fig. 2.

PLATE II.

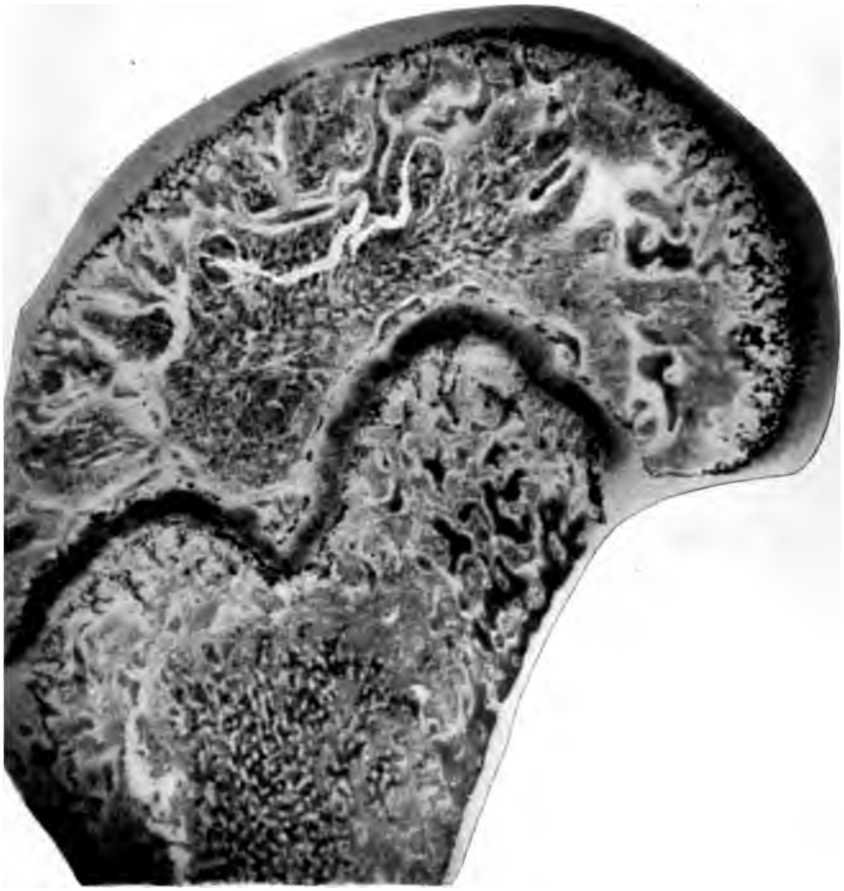

FIG. 3.

PLATE III.

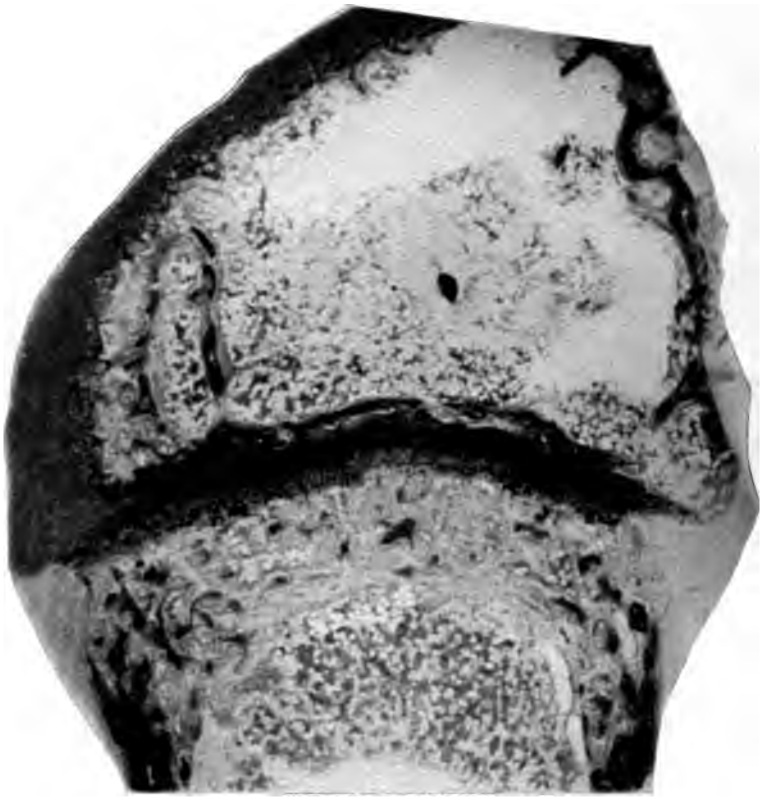

Fig. 4.

PLATE IV.

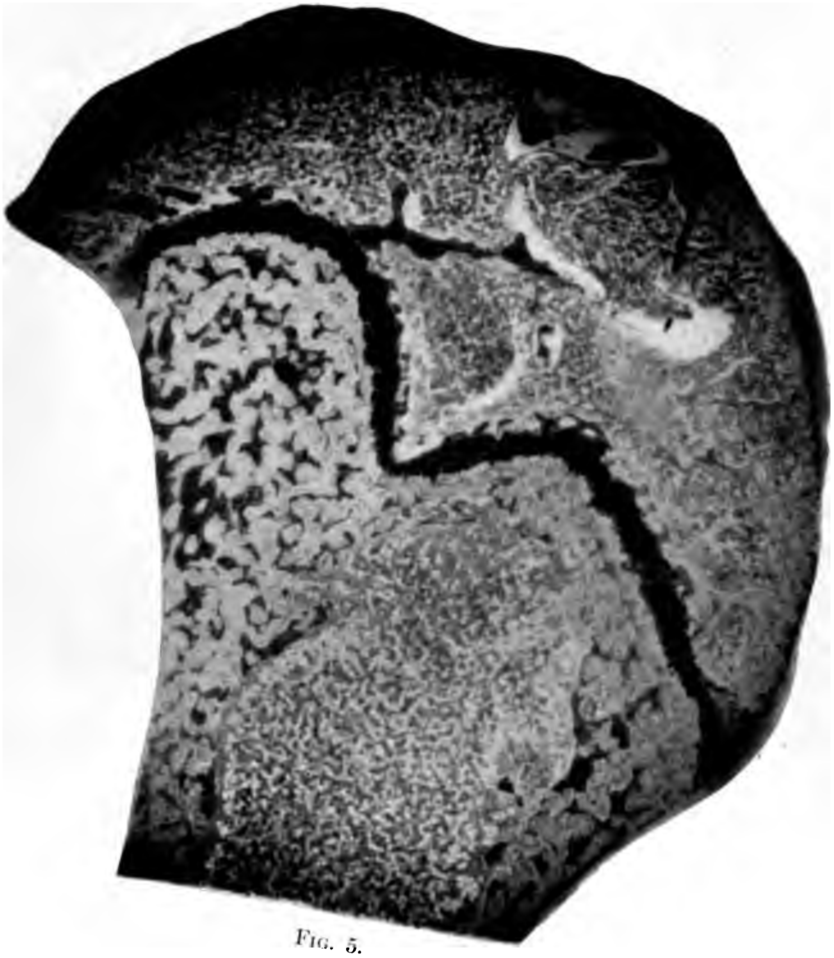

Fig. 5.

PLATE V.

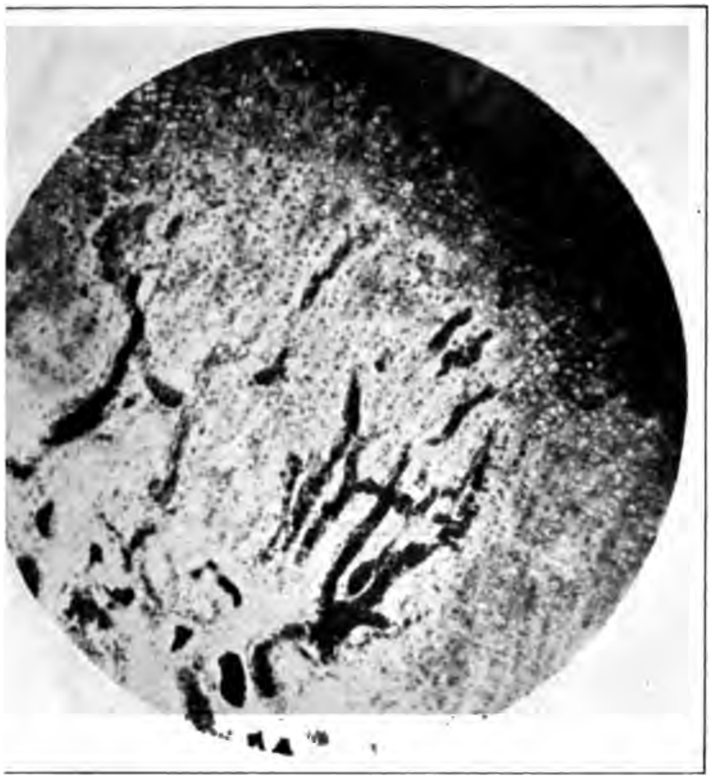

Fig. 6.

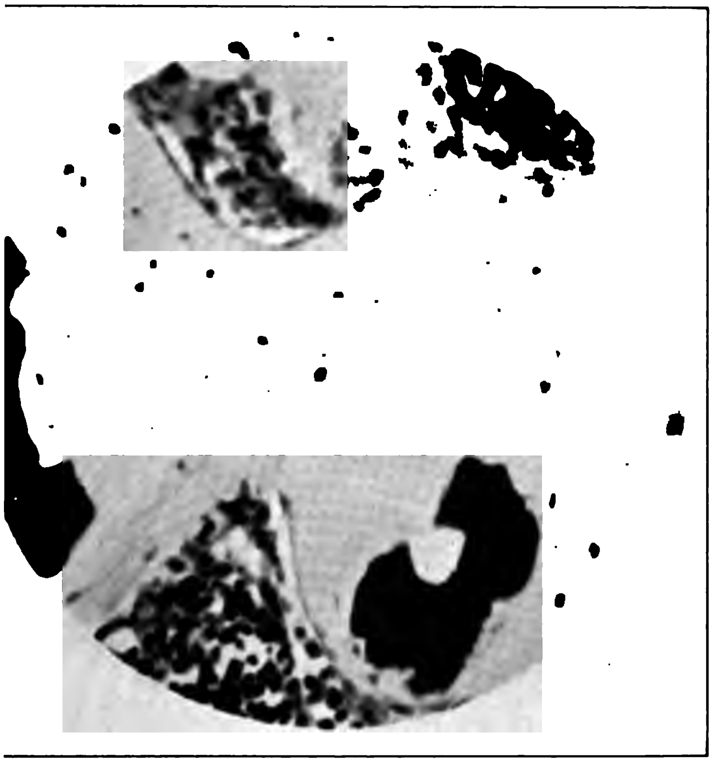

Fig. 7.

PLATE VI.

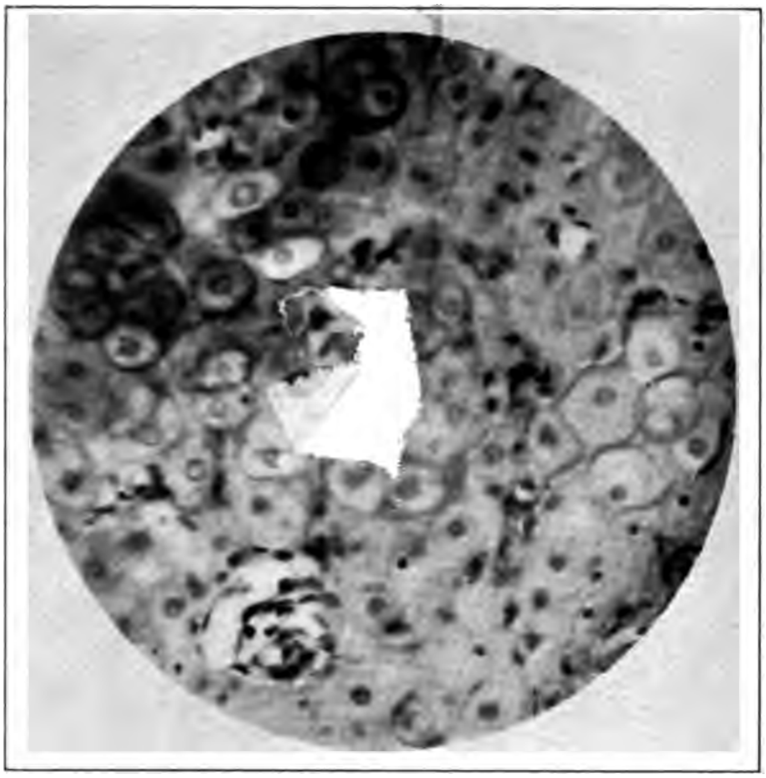

FIG. 8.

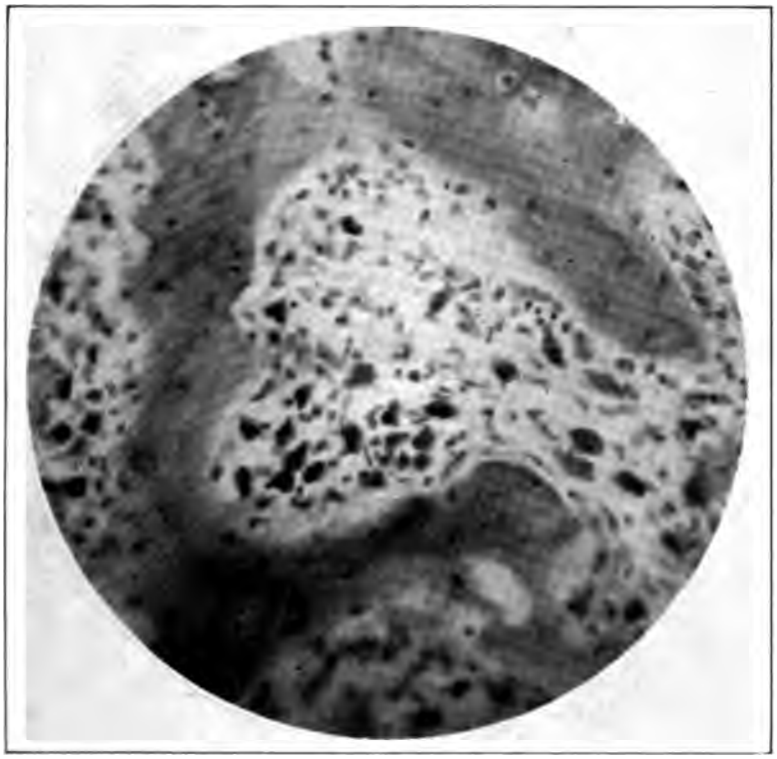

FIG. 9.

PLATE VII.

IS THERE MORE THAN ONE KIND OF RICKETS?*

P. G. SHIPLEY
From the Department of Pediatrics, the Johns Hopkins University
BALTIMORE

AND

E. A. PARK
From the Department of Pediatrics, Yale University
NEW HAVEN, CONN.

E. V. McCOLLUM AND NINA SIMMONDS
BALTIMORE

For at least two years our attention has been attracted to the possibility of the existence of more than one kind of rickets. That is, under the head of rickets may be included more than one kind of disturbance of the mineral metabolism of the body which manifests itself in a characteristically defective growth and calcification of the bones. The possibility was suggested by a series of clinical observations concerning the association of rickets with other diseases or pathological conditions and certain peculiar manifestations of rickets itself in some children. Inasmuch, however, as the idea of the duality or multiplicity of rickets rested solely on clinical observations which, at most, were only of a suggestive nature, we have never felt justified in presenting it as a definite hypothesis. But, recently, our experiments with the rat have yielded results which strongly suggest that there may be two distinct forms of the disease.

EXPERIMENTAL EVIDENCE

In previous publications[1] we have described certain defective diets which, when fed to the young rat, produce marked disturbances in the growth and calcification of the skeleton. The diets in question were alike in being insufficiently supplied with a factor or factors present in cod liver oil but differed from each other considerably in the composition of their mineral fraction, chiefly as regards the calcium and phosphorus. They may be divided into two groups according to the relative amounts of those two elements present. In one group the phosphorus was at a low level but the calcium at or above the level which would be optimal if all other dietary factors were satisfactory. In the other group the calcium was at an extremely low level but the phosphorus was not far from the optimal.

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* From the Laboratory of the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University.

[1] Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmonds, Nina: Studies on Experimental Rickets. III. A Pathological Condition Bearing Fundamental Resemblances to Rickets of the Human Being Resulting from Diets Low in Phosphorus and Fat-Soluble A: The Phosphate ion in Its Prevention, Johns Hopkins Hosp. Bull. **32**:160 (May) 1921. McCollum, E. V.; Simmonds, Nina; Shipley, P. G., and Park, E. A.: VI. The Effects on Growing Rats of Diets Deficient in Calcium, Am. J. Hygiene **1**:492 (July) 1921. McCollum, E. V.; Simmonds, Nina; Shipley, P. G., and Park, E. A.: VIII. The Production of Rickets by Diets Low in Phosphorus and Fat-Soluble A, J. Biol. Chem. **47**:507 (Aug.) 1921. Shipley, P. G.; Park, E. A.; McCollum, E. V., and Simmonds, Nina: XVII. Am. J. Hygiene **2**: 1922.

When the diets of the first group (those in which the phosphorus was deficient and the calcium phosphate ratio high) were fed to young rats living under ordinary laboratory conditions (room light), a pathologic condition of the skeleton developed, identical in all essential particulars with that seen in the rickets of human beings. The costochondral junctions were greatly enlarged; in some animals the thoracic wall was sunken at the sites of the costochondral junctions; the shafts of the ribs were fractured (Fig. 1). The long bones of the extremities were enlarged at the ends and could be cut easily. Between shaft and cartilage was a yellowish-white zone from two to three millimeters deep—the rachitic metaphysis. The proliferative cartilage extended in irregular prolongations toward the shaft. Calcium deposition in the cartilage was entirely lacking or extremely defective. The intermediate zone between cartilage and shaft was of a composition identical with that found in the metaphysis of the bones of rachitic children. It was composed of: cartilage in all stages of metaplasia or degeneration into a material indistinguishable from the osteoid; trabeculae consisting of osteoid; blood vessels; marrow elements; scattered, irregular deposits of calcified material encased in osteoid; and connective tissue. These were intermingled in a disorderly manner. The trabeculae of the shaft were surrounded by broad investments of osteoid (Figs. 2, 3 and 11).

When the diets of the second group (those in which the calcium was deficient, the phosphorus at a level not far from the optimal and the calcium-phosphate ratio low) were fed to rats kept under ordinary laboratory conditions (room light), a diseased condition of the skeleton developed which also bore marked resemblances to the lesions found in the rickets of human beings. The gross deformities caused by the second group of diets were as great or greater than those caused by the first group and corresponded exactly to the deformities found in rachitic children. The thorax was even more deformed than in the rats fed the diets of the first group; it was flattened from side to side and marked by deep grooves which followed the costochondral junctions; the angular deformities produced at the junction of the costal cartilages and the shafts projected into its interior; the costochondral junctions were enlarged and greatly distorted; fractures in the shafts of the ribs were especially numerous (Fig. 4). The lower ends of radius and ulna were enlarged as were also the ends of all the long bones of the extremities and the bones were extremely soft and weak. Between the cartilage and the shaft was a white intermediate zone one to three millimeters deep. Microscopic examination showed that the cartilage was entirely or nearly free from calcium and was invaded in an irregular manner by the vascular elements of the shaft. In consequence, the cartilage extended toward the shaft in irregular prolongations. The cells of the cartilage in proximity to the shaft showed evidences of degeneration and metaplasia. The intermediate zone was composed of cartilage in a more or less degenerated state, osteoid trabeculae, blood vessels surrounded by marrow elements, and a few deposits of calcium for the most part situated near the periphery and connective tissue. The trabeculae of the shaft were bordered by rather broad zones of osteoid. A loosely arranged fibrous tissue invested many of the trabeculae and in those places in which it filled in the

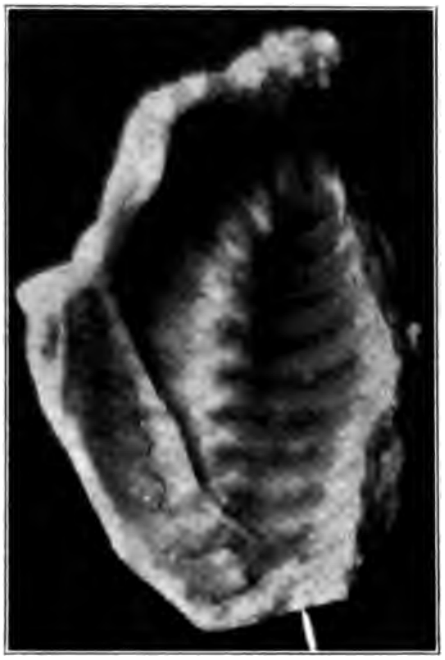

Fig. 1.—Pleural aspect of thorax of a rat which had developed rickets on diet 3,143 high in calcium and proportionately low in phosphorus.

spaces between the trabeculae, it gave rise to histologic pictures which closely resembled those furnished by the fibrous marrow in the rickets of human beings (Figs. 5, 7, 8 and 10).

The pathologic condition induced in the bones by the diets of the second group did not, however, correspond at all points to that usually found in the human subjects of the disease. The metaphysis was composed in larger part of osteoid trabeculae. Though these osteoid trabeculae

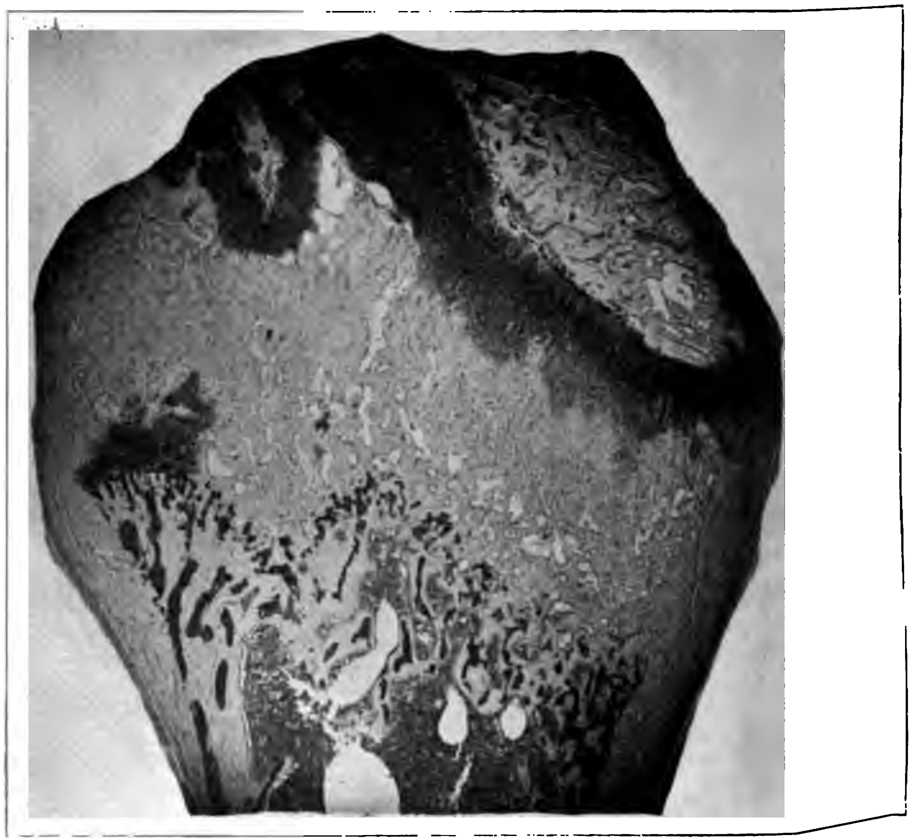

Fig. 2.—Microphotograph of a bone showing low phosphorus rickets. Note the lack of calcification of the cartilage, the very wide metaphysis, and the osteoid tissue surrounding the calcified trabeculae of the shaft.

were free from calcium deposition, they nevertheless retained a certain semblance of orderly arrangement. The osteoid zones about the trabeculae were not always so broad as in the rats on the diets of the first group, though they were quite as broad as the osteoid borders in the bones of rachitic children (Figs. 6 and 7). Cells evidently lerived from the fixed tissues with large basophilic granulations were iumerous in the immediate vicinity of the trabeculae (Fig. 9). Resorpive activity was exceedingly marked.

The abnormal condition of the skeleton produced by the faulty iets of the second group corresponded to that found in human beings ·ith rickets in all fundamental respects, namely, the degenerative and ietaplastic changes of the cartilage, the complete absence or defective iaracter of the calcification of the cartilage, an abnormal amount of steoid production, irregular invasion of the cartilage, and the producion of a rachitic intermediary zone. While one of the diets in question diet 2638) did not give absolutely constant results in all animals, in ome it produced a pathologic condition corresponding to that found in ·ickets of human beings even in its minor details.

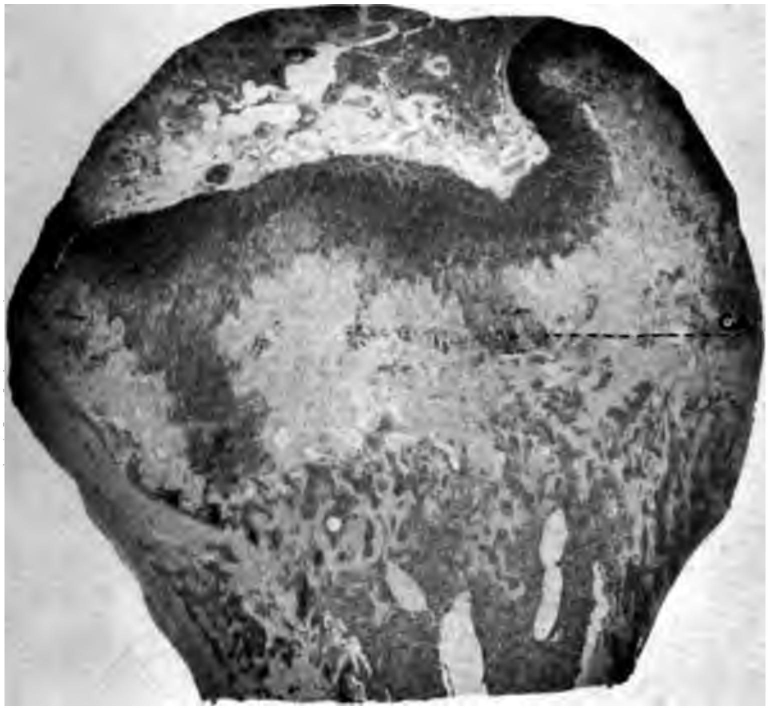

Fig. 3.—High calcium rickets in an animal on diet 3,133. This diet contins 2 per cent. added calcium carbonate instead of 3 per cent., as in diet ,143. Note the linear deposit of calcium in the metaphysis, which marks an bortive attempt to heal.

DISCUSSION

Our experiments make it clear that, when the rat is depri certain active light rays and an unidentified factor contained in co oil, a pathological condition corresponding in all fundamental re to rickets in human beings can be produced through the diet i ways: (1) by diminishing the phosphorus and supplying an o amount or an excess of calcium, or (2) by reducing the calciu maintaining the phosphorus at a concentration somewhere ne optimum. In the first the calcium: phosphate ratio is large, i second small. We are certain that in the human being sin deprived of light and the unidentified factor it would also be p to produce true rickets through a manipulation of the calcium and phate of the diet in the two ways mentioned.

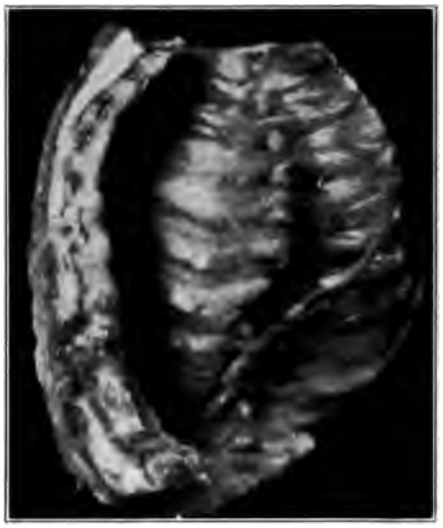

Fig. 4.—Pleural aspect of thorax of a rat which had developed low rickets on diet 2,638, which is high in phosphorus relatively, and calcium.

As a result of our experiments we are led to believe that the two main kinds of rickets. One is characterized by a normal or normal blood calcium and a low blood phosphorus (low phos rickets); the other by a normal or nearly normal blood phos but a low blood calcium (low calcium rickets). The investig of Howland and Kramer[2] and of Kramer, Tisdall and Howlan the calcium and phosphorus content of the blood serum in rickets and tetany have given suggestive evidence in support of this idea. These observers found that in children suffering from rickets alone, the phosphorus of the blood serum is low, and the calcium not far removed from the normal; in children suffering from manifest tetany complicating rickets, on the other hand, the calcium is low but the phosphorus not far removed from normal.

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[2] Howland, J. and Kramer, B.: Calcium and Phosphorus in the Se Relation to Rickets, Am. J. Dis. Child. **22**:105 (Aug.) 1921.

[3] Kramer, B.: Tisdall, F. F., and Howland, J.: Observations on I Tetany, Am. J. Dis. Child. **22**:431 (Nov.) 1921.

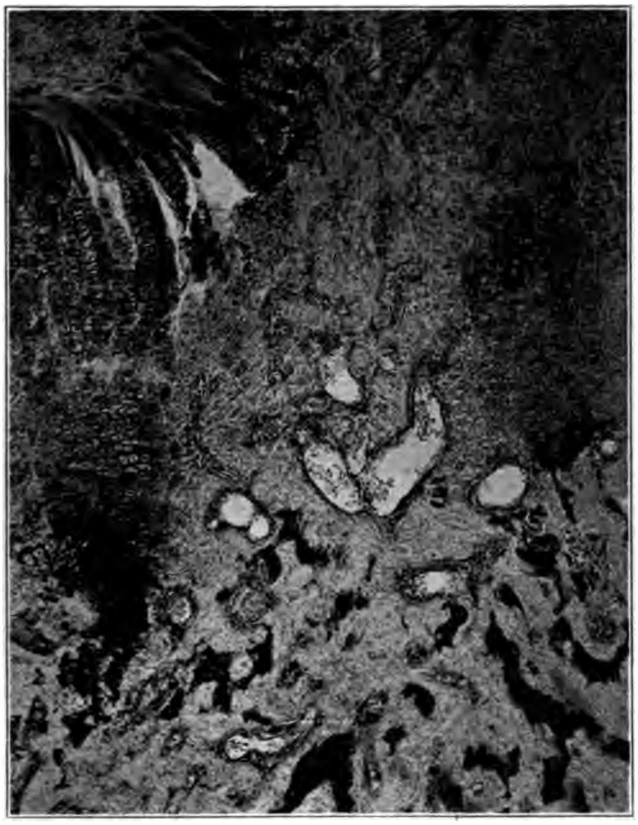

Fig. 5.—Low power microphotograph showing low calcium rickets. The cartilage is extremely irregular, and many cells have undergone metaplasia into osteoid tissue. There are long tongues and islands of cartilage to be seen in the metaphysis, which contains almost no calcified bone. Magnification × 70.

Rickets and Tetany.—As is well known, tetany complicates certain cases of rickets but not by any means all. Cases of rickets, even very severe rickets, exist in which tetany does not supervene, and, in all likelihood, never will. In other cases of rickets tetany either in the manifest or in the latent form is present for weeks and numerous observers have pointed out that it is with the less severe forms of rickets that tetany usually allies itself. Furthermore, experience has shown that a cure in rickets accompanied by tetany is most easily accomplished through the administration of calcium and cod liver oil or calcium and heliotherapy, whereas in rickets uncomplicated with tetany the cure is most readily accomplished by means of the administration of cod liver oil or heliotherapy with or without phosphate. The chemistry of tetany, its occurrence in some cases of rickets but not in all, and the fact that the successful form of treatment differs with the presence or absence of tetany, rather strongly suggest that rickets with tetany and rickets without tetany are distinct forms of metabolic disturbance affecting the skeleton.

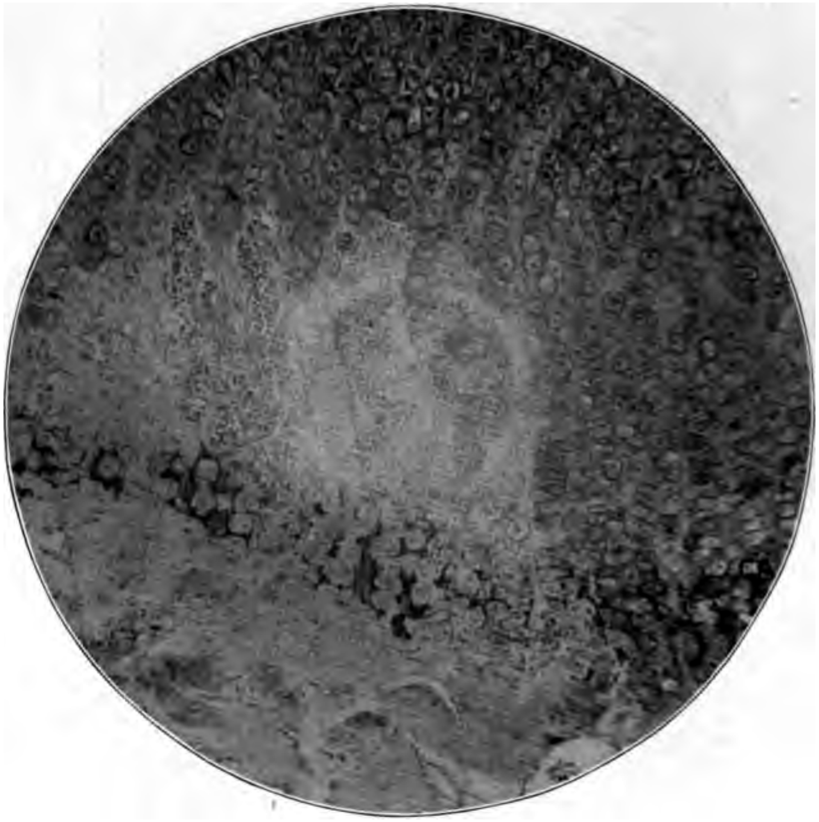

Fig. 6.—Microphotograph showing detail in the metaphysis of the bone (Fig. 3). Note the invasion of the cartilage by blood-vessels, its prolongation into the metaphysis, and the line of calcification (Ca) in the metaphysis which marks the occurrence of an aborted healing process.

Recently Huldschinsky[4] has advanced the view that tetany is othing more or less than a symptom complex of healing rickets. Vhile it is possible for tetany to occur with a healing rickets, clinical bservation alone is enough to show that tetany does not ordinarily ave that relationship to the disease. We have repeatedly seen chilren with rickets of the greatest severity undergo treatment with cod ver oil and have followed the healing process in the bones by means f the roentgen ray. None of the children ever showed the slightest idence of tetany during the process of cure.

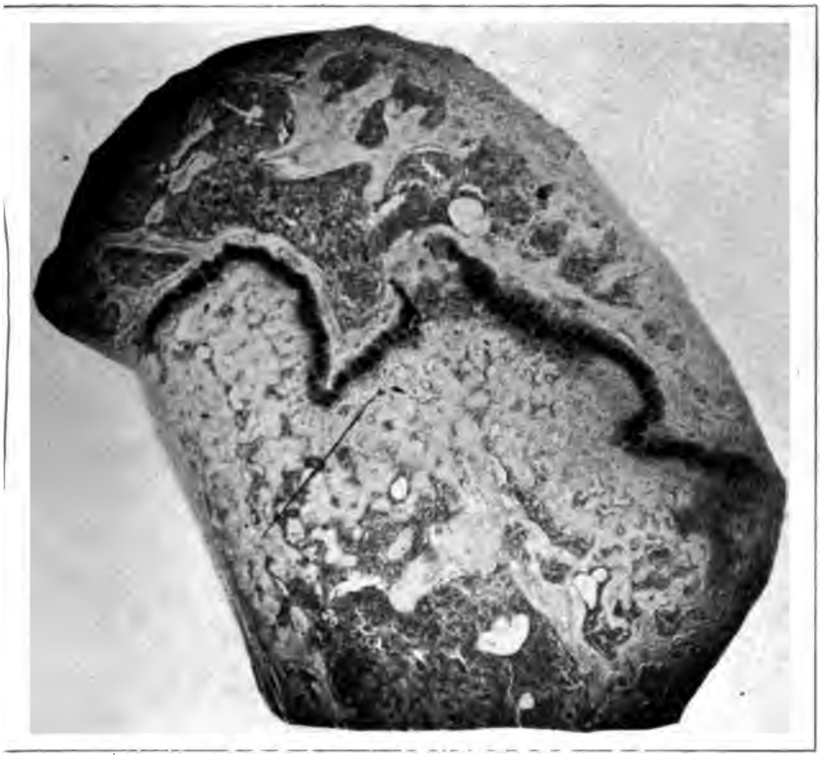

Fig. 7.—Low calcium rickets in the bone of an animal on diet 2,638.

It is undoubtedly true, however, that in some cases of rickets ccompanied by tetany the roentgen ray shows deposition of lime salts t the cartilage-shaft junctions of the long bones of the extremities. The reason for this, according to the present trend of our investigaions, is in many instances as follows: In the low calcium and the ow phosphorus forms of rickets, calcium: phosphate ratios exist in the blood which do not permit at all or permit only to a very limited extent the deposition of calcium phosphate in the bones. Between the low calcium form of the disease and the normal condition, on the one hand, and the low phosphorus form and the normal condition on the other, are innumerable gradations, presumably characterized by calcium: phosphate ratios in the blood, very probably oscillating ratios, which permit calcium phosphate deposition in varying degree, from the most fragmentary to almost complete calcification (Figs. 3 and 6). In connection with both the low calcium and the low phosphorus forms of rickets, therefore, there are borderline states of the disease in which the calcium: phosphate ratios are such as to permit calcium phosphate deposition to take place irregularly or intermittently in the bones. With the borderline cases, in particular those connected with the low calcium form of the disease, we believe tetany to be frequently associated.

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[4] Huldschinsky, K.: Die Beeinflussung der Tetanie durch Ultravioletlicht, Ztschr. f. Kinderh. **26**:5, 1920.

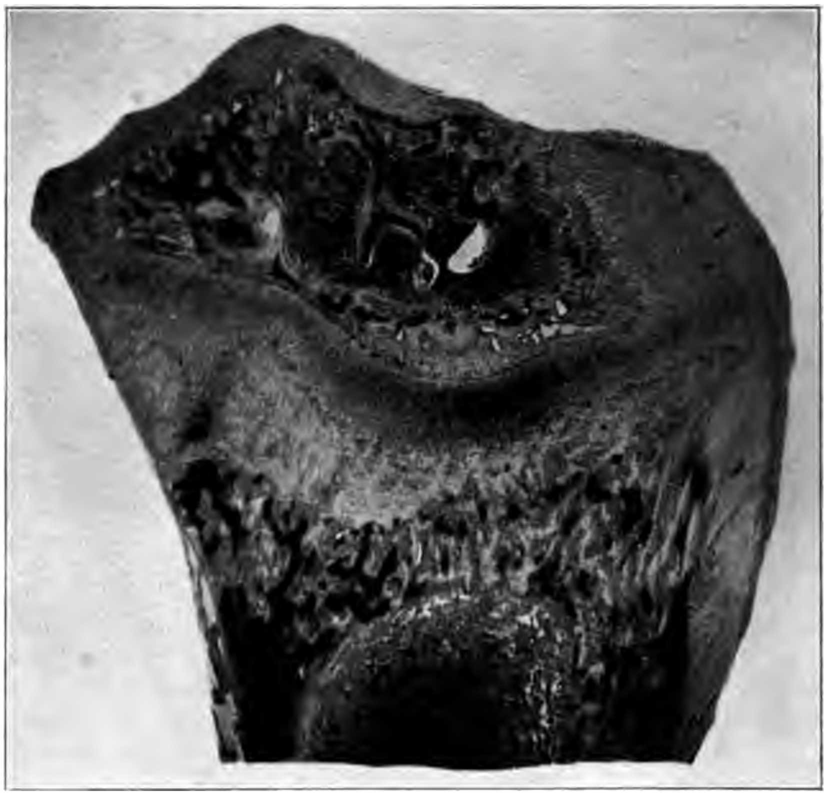
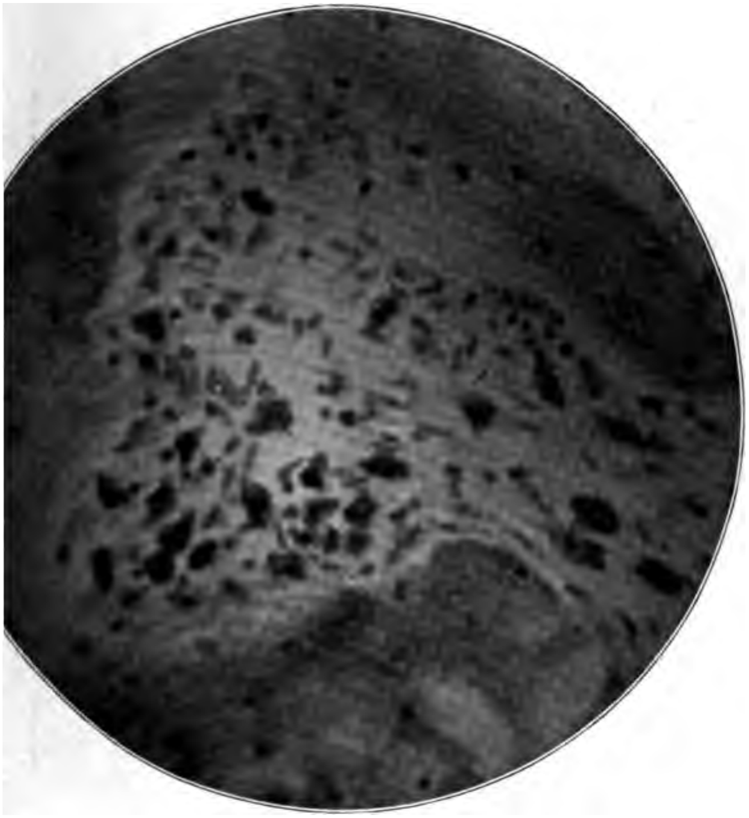


Fig. 8.—Low calcium rickets from a rat on diet 3,041. This diet contains the same amount of calcium as diet 2,638, but the phosphate content of the diet is slightly higher.

r experiments indicate that deposits of calcium salts at the seal line may occur as a result of (1) healing in the low phos- form of rickets, (2) healing in the low calcium form and (3) onstantly occurring or recurring phenomenon in the borderline of the disease. With the first we believe that tetany is rarely ted and then, usually certainly, only in the latent form; with :ond, tetany may be associated; with the third we believe it to quently associated.



9.—High power microphotograph showing basophil connective tissue ar the trabeculae of bone in the shaft of an animal with low calcium diet 3,141.

:ording to present knowledge, the relationship between tetany :kets appears to be as follows: Tetany is an expression on the f the nervous tissues of an insufficiency of the calcium ion; is an expression on the part of the skeleton of disturbed ıs between the calcium and phosphate ions. The reason that is so frequently associated with rickets is because rickets is a disease in which the calcium ion in the body tissues and fluids is subject to wide variations. Tetany occurs independently of rickets, jus as rickets occurs independently of tetany. Since tetany may occ with the low phosphorus form of rickets, it does not serve to mark one form of rickets from the other. Tetany is essentially associat however, with the low calcium form, and, for all practical purpo the low calcium form of rickets is the rickets of tetany.

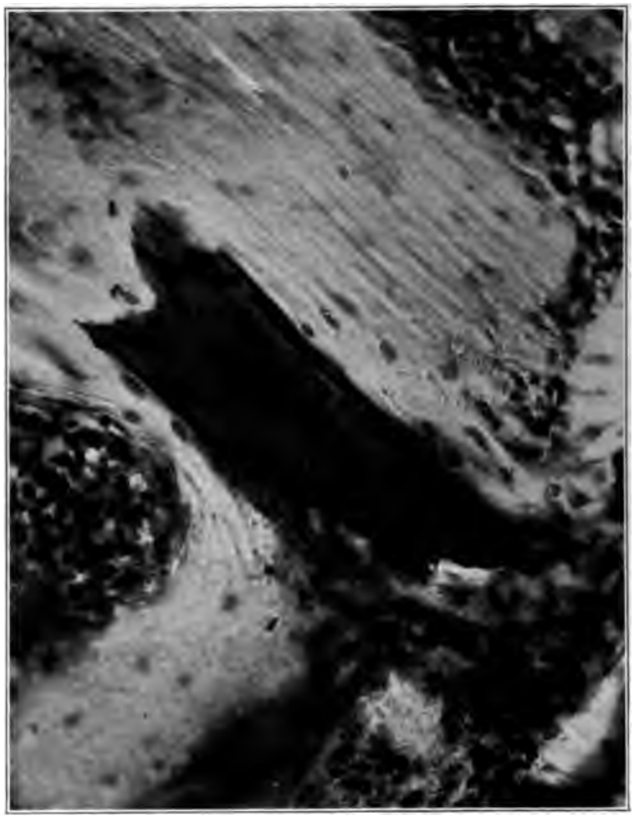


Fig. 10.—High power microphotograph to show the osteoid tissue fro case of low calcium rickets. Magnification × 375.

Rickets and "Renal Dwarfism."—Recently Barber, Patterson a others have described children and young adults who were undersize had rachitic deformities, and at the same time were the subjects severe forms of chronic interstitial nephritis or developmental defec of the kidney. To the syndrome of symptoms which they exhibite Barber gave the name "renal dwarfism." The age of one of Patte son's patients was only one year and 9 months, but all others wer between 6 and 20 years of age. If the infant described by Patterson be excepted, the height was from 6 to 16 inches under the average for the age, and the rickets was peculiar in that it was a late and gradual development (rachitis tarda). The disturbance of renal function in some of the cases was known to be extreme.

We have seen a child, aged $3\frac{1}{2}$ years, whose case belongs in this category. The rachitic deformities were extreme. Moreover, the changes in the bones, as revealed by the roentgen ray and histologic examination, were most extensive and differed from the changes ordinarily found in rickets. The proliferative cartilage was most irregular and the calcification defective. In the deep metaphysis were large islands of cartilage bordered on one or more sides by dense calcium deposits. Those islands of cartilage gave to the metaphysis, as seen in the roentgenogram, a honeycomb appearance. The trabeculae were thin and the osteoid borders, comparatively speaking, narrow. Surrounding the trabeculae and islands of cartilage and lying between them was a loose fibrous tissue. Obviously, for a long time the pathologic condition in the bones had been in a state of flux between healing and relapse. The specific gravity of the urine was low and

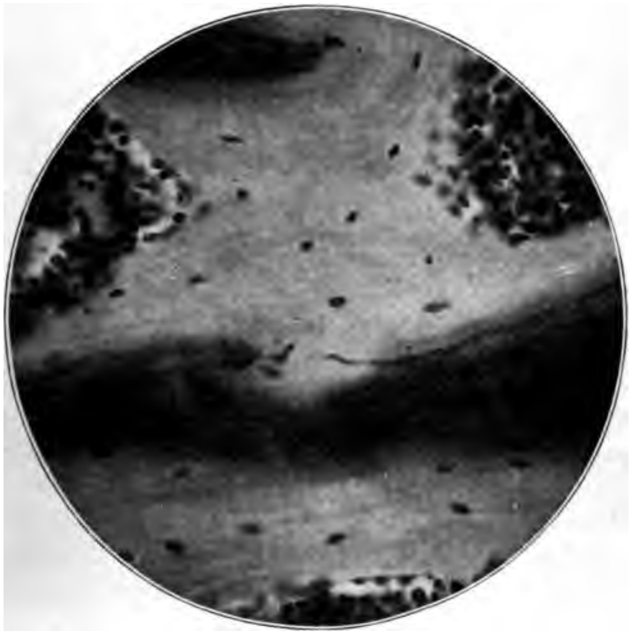


Fig. 11.—This picture shows an osteoid and calcified bone in a trabecula from a case of low phosphorus rickets. Animal was on our diet 3,143. Same magnification as in Figure 10.

appeared to be fixed. Albumen was constantly present. Several attacks which were thought to be uremic acidosis occurred. After death the kidneys were found to be exceedingly small and were defectively developed.

The histologic condition found in the bones of the infant described by Patterson appears to have been exactly similar.

It seems possible to us that the rickets which develops in the subjects of "renal dwarfism" has its origin in the extreme functional disability of the kidney and may belong to the low calcium form of the disease, i. e., that it may be dependent on an inability of the kidney properly to excrete phosphate. If our suggestion should prove to be correct, a true "renal rickets" exists and rickets under certain conditions may have an endogenous origin.

The Rickets Occurring in Children Suffering from the Severer Secondary Anemias.—Some infants develop a marked anemia which is apparently independent of concurrent infection. In many instances, it seems probable that this anemia is of dietetic origin, since breast feeding, or a diet almost entirely composed of cow's milk, has been unduly prolonged. The blood picture is that of a secondary anemia but sometimes corresponds to the picture generally known as von Jaksch's anemia. The children showing this abnormal condition of the blood regularly show rickets. The rachitic deformities are, however, somewhat different from those ordinarily seen and are fairly uniform. The frontal and parietal eminences are large and the regions of the sagittal and coronal sutures often appear sunken. The enlargement of the costochondral junctions and the ends of the long bones of the extremities is moderate and the degree of involvement at the cartilage-shaft junctions does not seem to be advanced. The involvement of the skull appears to be out of proportion to the involvement of the bones of the extremities or of the thorax.

We can form no definite idea concerning the nature of the disturbance in the metabolism which causes the rickets in the children suffering from severe secondary anemia. Probably the rickets and the anemia have a common origin, or are connected in some way. In any event it is interesting that in a recent series of experiments Happ[5] was able to produce the most severe anemia and rickets in the rat by feeding certain diets extremely low in calcium. The experiments of Happ might indicate, therefore, that the rickets accompanying the severe so-called "alimentary" anemias belong to the low calcium form of the disease. Children showing the peculiar combination in question, however, failed to show evidences of tetany. It seems to us possible that the rickets accompanying the alimentary anemias may represent

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5. Happ, W. M.: In press (Personal communication).

a somewhat different kind of disturbance in the mineral metabolism from the ordinary forms of the disease. Evidently another tissue, the blood, which is not regularly affected in rickets, is included in the metabolic disturbance.

We are prepared to find the etiology of rickets as varied as the etiology of tetany. Tetany was once considered to be an entity. Now, it is necessary to regard it as a symptom complex which can be produced experimentally in a variety of ways and which occurs naturally in several conditions. Tetany may be produced by excision of the parathyroid glands, by the injection into the blood stream of sodium phosphate or of sodium bicarbonate, by diets high in sodium phosphate and by the creation of gastric fistula which result in the loss of the gastric secretions. It occurs spontaneously in association with rickets and sometimes dilatation of the stomach. We should suppose that any influence which would result in the depression of the calcium or the phosphorus ions in the body fluids with the formation of the calcium: phosphate ratios favorable for the development of rickets if continued long enough would produce the disease and that there might be several such influences.

CONCLUSIONS

1. Our experiments make it clear that when the rat is deprived of certain active light rays and an unidentified factor contained in cod liver oil, a pathologic condition corresponding in all fundamental respects to rickets in human beings can be produced through the diet in two ways: (1) by diminishing the phosphorus and supplying the calcium in optimal quantities or in excess, or (2) by reducing the calcium and maintaining the phosphorus at a concentration somewhere near the optimum.

2. We believe it to be certain that in the human being, similarly deprived of light and the unidentified factor, it would also be possible to produce true rickets through an adjustment of the calcium and phosphorus of the diet in the two ways mentioned.

3. As the result of our experiments we are led to believe that there are two main kinds of rickets. One is characterized by a normal or nearly normal blood calcium and a low blood phosphorus (low phosphorus rickets); the other by a normal or nearly normal blood phosphorus but a low blood calcium (low calcium rickets).

4. If the hypothesis just stated is correct, the relation of tetany to rickets would appear to be as follows: Tetany is essentially an expression on the part of the nervous tissues of an insufficiency of the calcium ion; rickets is essentially an expression on the part of the skeleton of disturbed relations between the calcium and phosphate ions of the body fluids. Tetany is frequently associated with rickets

because rickets is a disease in which the calcium ion in the body tissues and fluids is subject to variations. Tetany occurs independently of rickets, just as rickets occurs independently of tetany. Since tetany may occur with the low phosphorus form of rickets, it does not serve to mark off one form of rickets from the other. Tetany, however, is essentially associated with the low calcium form of rickets, and, for all practical purposes, the low calcium form of rickets is the rickets of tetany.

5. It seems possible that the etiology of rickets may be as varied as the etiology of tetany. Our experiments lead us to suppose that in the absence of certain active light rays and an unidentified dietary factor contained in cod liver oil, any influence which would result in the depression of the calcium or phosphate ions in the body fluids with the formation of calcium: phosphate ratios favorable for the development of rickets would ultimately produce the disease. We are prepared to think that there may be several such influences.

6. For some time, as the result of clinical observations concerning curious associations of rickets with other pathologic conditions and certain peculiar manifestations of the disease in some children, our attention has been attracted to the possibility of the existence of more than one kind of rickets. In the light of our experiments we suggest the possibility that there may be a true renal rickets and that the rickets accompanying the alimentary anemias may represent a somewhat different kind of disturbance in metabolism from that which is present in the ordinary forms of the disease.

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[From THE JOHNS HOPKINS HOSPITAL BULLETIN, April, 1922, XXXIII, Page 125.]

STUDY ON EXPERIMENTAL RICKETS. XIX.

THE PREVENTION OF RICKETS IN THE RAT BY MEANS OF RADIATION WITH THE MERCURY VAPOR QUARTZ LAMP

By G. F. POWERS and E. A. PARK
P. G. SHIPLEY
E. V. MCCOLLUM and NINA SIMMONDS

(*School of Hygiene, Johns Hopkins University, Baltimore, Md.*)

In a previous article[1] we showed that when rats were [125] placed on a rickets-producing diet (diet 3143) they did not develop rickets if they were exposed to direct sunlight.

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[1] The Prevention of the Development of Rickets in Rats by Sunlight. XI. P. G. Shipley, Dept. of Pediatrics, Johns Hopkins University, Baltimore, Md., E. A. Park, G. F. Powers, Dept. of Pediatrics, Yale University, New Haven, Conn., E. V. McCollum and Nina Simmonds, School of Hygiene, Johns Hopkins University, Baltimore, Md. *Proc. Soc. for Exper. Biol. and Med., Oct. 19, 1921.*

STUDIES ON EXPERIMENTAL RICKETS. XIV. The Prevention of the Development of Rickets in Rats by Sunlight. P. G. Shipley, Dept. of Pediatrics, Johns Hopkins University, Baltimore, Md., E. A. Park, G. F. Powers, Dept. of Pediatrics, Yale University, New Haven, Conn., E. V. McCollum and Nina Simmonds, School of Hygiene, Johns Hopkins University, Baltimore, Md. *Jour. Am. Med. Assn., Jan. 21, 1922, Vol. 78, pp. 159-165.*

[126] As the experiments progressed, certain differences in the behavior of the two groups of animals were observed. The rayed animals were extremely active, the contrast between the two groups becoming more and more marked and reaching its maximum in the second third of the experimental period. Whenever any one came near the cage in which these animals were, a constant scurrying to and fro was almost always the striking feature observed. Not only were the control animals markedly less active, but their gait was distinctly waddling in character after the third or fourth week of the experiment. The disability of the control animals was well illustrated by the fact that toward the end of the experiments seven of the animals got out of their cage and were all recovered with relatively little difficulty.

The rayed animals always seemed to be hungry and, although the food given to each group was not measured, it was obvious that the amount consumed by the animals receiving mercury lamp radiations greatly exceeded that consumed by those in the control group. Increased appetite, then, was a striking characteristic of the rayed animals.

One of the rayed animals gave birth to six young on the thirty-seventh day of the experiments.

The autopsies revealed many differences in the animals. As shown in the following table, the measurements corroborate the observations made as to the better physical development of the animals exposed to the radiations from the mercury vapor quartz lamp.

	Control Animals (8)	Radiated Animals (9)	Per cent Increase in Radiated over Controls
Average Weight	140 grams.	157 grams.	12
Average Nose-Tail Length	31.6 cm.	34.5 cm.	9
Average Nose-Rump Length	15 cm.	16.1 cm.	7
Average Tail Length	16.6 cm.	18.4 cm.	10

The hair of the rayed animals was noticeably thicker and coarser than that of the control animals. The hair on the backs of the albino rats exposed to ultra-violet rays was tinted a slight lemon yellow. There was no enlargement of the epiphyseal junctions in the rayed ani-

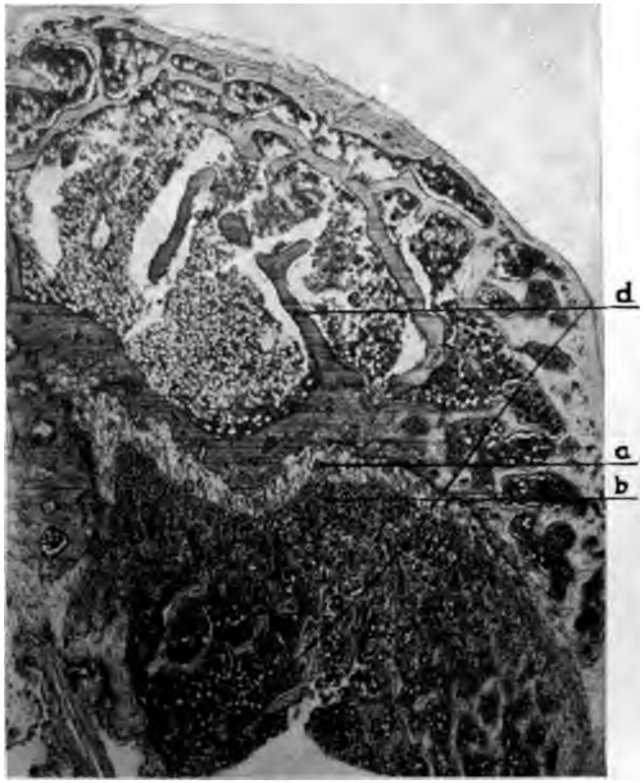

FIG. 1

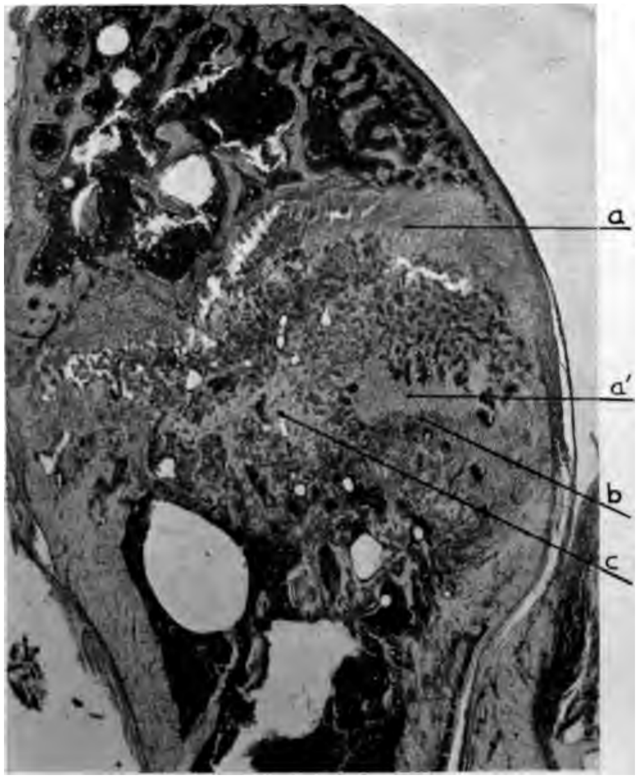

FIG. 2

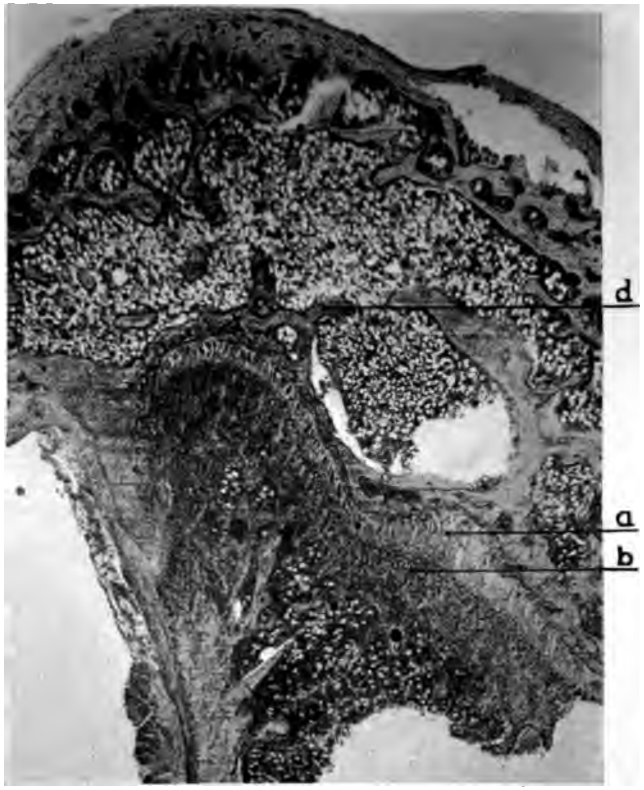

FIG. 3

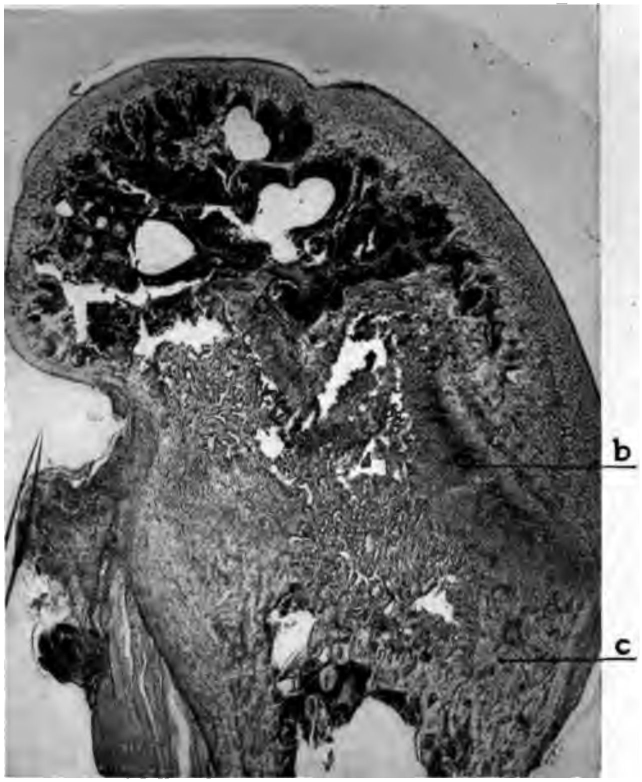

FIG. 4

mals. On opening the bodies of the rayed animals the [126] most striking feature was the great amount of fat deposited in the subcutaneous tissue and in the peritoneal cavity. In some animals this deposition was tremendous. It occurred in some of the control rats but was never great. The muscular development of the rayed rats was better than that of the control animals. There were no deformities of the thorax and no gross evidences of rickets in the long bones of the rayed animals.

The control rat (16Y), autopsied thirty-eight days after the beginning of the experiment, showed the typical picture of advanced rickets. The incisor teeth were brittle, [127] the molar teeth were loosened; there was enlargement of the knees and wrists; the costo-chondral junctions were enlarged and bent inwards, and there were numerous fractures of the ribs. The tibiae and femora on section showed the typical gross picture of rickets.

The other control animals, autopsied about four weeks later, showed less marked but equally definite signs of rickets. The teeth were only slightly more easily fractured than normal. There was moderate enlargement of the epiphyses of the long bones of the extremities. The costo-chondral junctions were enlarged but there was little deformity of the chest and no well marked fractures of the ribs. The bones of the extremities cut with diminished resistance and in the femora of some there were gross evidences of rickets on section. The viscera showed no pathological changes. The amount of subcutaneous and peritoneal fat was much less than in the rayed animals. The muscles of the control animals were relatively poorly developed.

Microscopic examinations of the bones were confirmatory of the gross findings. In sections of the long bones of the rayed animals there were no evidences of rickets. The line of demarcation between the cartilage and shaft was sharp and clear cut. The deposition of calcium in the provisional zone of cartilage was heavy and complete. The trabeculae showed only the thin margin of osteoid

[127] which is seen in healthy, growing animals and which must be regarded as physiological.

The sections of the long bones of the control animals without exception showed rickets, but in some specimens the rachitic process was moderate in severity. The trabeculae in all sections were surrounded by broad osteoid borders and the calcification of the provisional zone of cartilage was fragmentary and exceedingly irregular. The degree of rickets usually found in rats on this diet is more severe than is shown in some of the specimens from these animals. This is probably explained by the fact that the animals were fifty days old when placed on the diet and were in some instances one hundred and fourteen days old when killed. During the period covered by the experiments, therefore, activity in growth was declining and with it there was a decline in the activity of the rachitic process.

DISCUSSIONS

From this experiment it is possible to say that rats fed on the rickets-producing diet (diet 3143) are protected from that disease by exposure to radiations from a mercury vapor quartz lamp. It is possible to say further that radiations from the mercury vapor quartz lamp affect not the skeleton alone but indeed the whole organism. While the development of a normal skeleton in the rayed animals is a "striking, visible and measurable" effect of the radiations on a single tissue, growth, good muscular development, storage of fat, improvement in the condition of the hair, stimulation of sexual development and reproductive power are evidences that the radiations have a favorable influence upon the animals as a whole. So far as we are able to discern, the action upon rats fed the rickets-producing diet (diet 3143) of the radiations of a mercury vapor quartz lamp in securing an efficient utilization of the substances which are directly or indirectly concerned with ossification and calcification and in promoting general bodily vigor is in no way different in respect to these matters from the action of cod-liver oil and of sunlight.

SUMMARY

1. The object of the experiments was to determine whether or not radiations from a mercury vapor quartz lamp prevent the development of rickets in the rat.

2. A diet was employed which at room light regularly gives rise to a disease identical in its essential features with rickets as seen in the human being. The diet was high in calcium, low in phosphorus and was insufficiently supplied with fat-soluble A. In other respects it was well constituted.

3. Nineteen rats were placed on the diet. Ten were exposed to radiations from a Hanovia "Alpine" mercury vapor quartz lamp for varying periods of time daily over a period of sixty-four days. Nine rats were kept under conditions of ordinary room light as control animals.

4. One of the control animals was killed after thirty-eight days; another after fifty-eight days and the remaining seven after sixty-four days. All of these animals showed gross and microscopic evidences of rickets.

5. The ten rats exposed to the radiations from the mercury vapor quartz lamp were killed after sixty-four days. These animals were free from rickets both grossly and histologically.

6. The beneficial effects of the radiations from the mercury vapor quartz lamp were not limited to the skeleton, since the condition of the rayed animals underwent a general improvement.

7. The effects of the radiations of the mercury vapor quartz lamp on the growth and calcification of the skeleton of the rat and on the animal as a whole seem to be similar to, if not identical with, those brought about by direct sunlight and by cod-liver oil.

DESCRIPTION OF PLATES

Figs. 1 and 2. Microphotographs of sections of femora of rats fed on diet 3143 and exposed to radiations from a mercury vapor quartz lamp. Note sharp demarcation between shaft and cartilage at metaphysis (a), the heavy calcification of provisional zone of cartilage (b), and the wide bony trabeculae without osteoid borders (d).

[127] Figs. 3 and 4. Microphotographs of sections of f
rats fed on diet 3143 and kept at room light. N
irregular metaphysis.

Fig. 3. a—Proliferative cartilage.
à—Islands of cartilage with (bFig.3) dep
calcium on the diaphyseal side.
c—Osteoid trabeculae.

Fig. 2. b—Cartilage is irregular and uncalcified.

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A RAPID AND ACCURATE METHOD FOR CALCIUM IN URINE.

BY ALFRED T. SHOHL AND FRANK G. PEDLEY.

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

(Received for publication, December 12, 1921.)

McCrudden's method (1, 2) for the determination of calcium in urine, at present probably the most accurate and satisfactory of all the calcium methods, is open to the serious criticism that it requires platinum dishes, and is laborious and time-consuming. Not infrequently it takes 24 hours to filter the urine and wash the precipitate. After filtration the ashing and weighing usually occupy an additional day, sometimes longer. McCrudden endeavored to shorten the method by titrating the precipitated calcium oxalate with potassium permanganate, but as the precipitate was contaminated with uric acid obtained too high readings. He states:[1]

> "I have concluded, therefore, if calcium oxalate, precipitated from urine, is contaminated with uric acid, as indicated by the reddish color of the precipitate, the calcium should not be determined by titration."

It is very questionable whether color is a good indication of the presence or amount of uric acid, since pure uric acid is colorless. The urinary uric acid, as is well known, depends for its coloration upon the presence of urinary pigments. Because of the above warning, and our experience, we have always relied on McCrudden's method only after ashing and weighing the calcium as calcium oxide. We felt that his method would be improved if we could avoid gravimetric analysis. Such a rapid clinical method for the determination of calcium in the urine is, therefore, desirable.

The method about to be presented, has the advantage of increasing the speed of the determination without appreciably decreasing the accuracy. The problem before us was to precipitate the calcium as calcium oxalate avoiding contamination with uric acid, and then to titrate the precipitate with potassium permanganate.

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[1] McCrudden (2), p. 194.

We first attempted to remove the uric acid by filtration after adding sufficient acid to convert the urates to uric acid and also to dissolve any calcium oxalate present. This is inadequate since considerable time is required and uric acid is very slowly and not quantitatively precipitated from urine. We also object to filtration, which McCrudden recommends for an initial procedure without adding acid, on the ground that any calcium combined with oxalic acid would be held back by the filter paper and lost.

We then searched for some solvent of uric acid with which the precipitate of calcium oxalate and uric acid might be washed on the filter or which would keep the uric acid in solution. Various solvents were tried. Hot sodium carbonate readily dissolved the uric acid but caused the readings to be much too low. Hexamethyleneamine was found to be very inefficient. Piperidine, a noted uric acid solvent, rendered the urine highly alkaline, dissolved the uric acid by virtue of its alkalinity, and was therefore inapplicable. No satisfactory solvent was found.

To eliminate the uric acid, the urine could be either dry ashed or ashed in the wet way following the Neumann method (3). Both these methods have been utilized in determining calcium. There is no objection to them except for the time and labor involved. We thought we might save time and avoid technical difficulties; and if we could destroy the uric acid by oxidation, we would accomplish in a short time, without evaporating the urine, the same purpose as ashing. Any substance which would reduce permanganate would be removed by oxidation.

We tried concentrated solutions of potassium permanganate on theoretical solutions containing calcium and uric acid with correct results. With urine, however, a surprising amount of this reagent was needed for complete oxidation. And this left large quantities of manganous ions, which, in the presence of oxalate ions, were difficult to hold in solution. It is not impossible that this method may be applicable, since manganous oxalate, like magnesium, is held in solution by ammonium chloride (4). We sometimes obtained an impure precipitate.

We then used ammonium persulfate and obtained excellent results (5). This reagent has the advantage of being relatively cheap, of being a strong uric acid-oxidizing agent, and an inefficient oxalate-oxidizing agent. In fact on theoretical grounds it is practically ideal for the purpose. When oxidation is complete, instead of manganous ions, we have sulfate and sulfite ions in solution, and these are not objectionable.

Ammonium persulfate is a white crystalline, deliquescent substance, which deteriorates on exposure to the air. When first put into solution it crackles with the evolution of a gas that smells like ozone. When added to the urine it froths. These properties we have taken as a rough index of its activity. A sample we obtained from Eimer and Amend was very satisfactory, but contained an appreciable amount of calcium. A blank should be run, therefore, for the calcium content. The reagent oxidizes best in an acid medium.

After the urine has been oxidized the calcium is precipitated as oxalate. The method as suggested by McCrudden may be followed.[3] He uses sodium acetate to regulate the acidity. We carry out the precipitation between definite limits of hydrogen ion concentration (6). We have found that the optimum lies between pH 4.8 to 5.2. Therefore, we use 1 drop of methyl red as an indicator. If the color fades rapidly we add a second drop of indicator solution. This indicator almost exactly covers the desired range of pH. It gives a red color when the acidity is as great or greater than pH 4.6, a yellow color when the acidity is less than pH 6.0, and an intermediate color between pH 4.8 to 5.4. This is the color desired. When one uses methyl red as an indicator one may use sodium acetate to decrease the acidity but it is not necessary. We use NH_4OH and bring the solution to the proper end-point, as shown by the color of the indicator. The important condition is to have the solution at the proper hydrogen ion concentration.

When the calcium has been precipitated as described above it is allowed to stand over night. (McCrudden shakes the mixture 10 minutes instead of allowing it to stand.) It is then filtered. Halverson and Schulz (7) have suggested filtering through a

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[3] McCrudden (2), p. 199.

Gooch crucible made with specially prepared and ignited asbestos This is unnecessary; for if the urine has been oxidized with persulfate it filters almost as readily as water. A good grade of hardened filter paper is easier to use. When several determinations are made at once it is practically as rapid as preparing the Gooch crucible and there is no chance of the filter's not retaining the precipitate. The precipitate is then washed back into the original flask and titrated with permanganate.

Reagents required.	
Ammonium persulfate.	Nitric acid (sp. gr. 1.42).
Oxalic acid, 2.5 per cent.	Sulfuric acid (sp. gr. 1.84).
Methyl red, 0.02 per cent in 50 per cent alcohol.	Ammonium hydroxide (sp. gr. 0.9)
Potassium permanganate, 0.05 N.	Sodium oxalate, Sörensen 0.05 N.

Details of the Method.

1. To 100 cc. of unfiltered urine in a 250 cc. Erlenmeyer flask add 5 cc. of concentrated HNO_3 or H_2SO_4, and one spoonful containing 3.0 to 4.0 gm. of ammonium persulfate. Insert a funnel in the flask to prevent spattering.

2. Boil and keep near the boiling point on a hot plate, or over a low flame, for 1 hour, or until reduction of the ammonium persulfate is complete, as evidenced by an absence of frothing when the flask is agitated. The solution at this point is pale green in color.

3. Add 10 cc. of 2.5 per cent oxalic acid.

4. Cool to room temperature.

5. Neutralize with ammonium hydroxide, using 1 drop of methyl red, as an indicator.

6. Cool to room temperature.

7. If the color is now red, the solution may be brought to the desired color by a few drops of ammonium hydroxide. pH 4.8 to 5.2.

8. Let stand over night (McCrudden shakes the mixture for 10 minutes instead of allowing it to stand).

9. Filter. Whatman No. 50 hardened filter paper, 12.5 cm., has been found satisfactory. Wash the precipitate and flask three times with distilled water, filling the filter two-thirds full each

time and allowing to drain. Break a hole in the filter paper, and wash back the precipitate into the original flask, first with distilled water, and then with hot dilute sulfuric acid, bringing the volume to about 100 cc.

10. Add 10 cc. of concentrated sulfuric acid, and heat to 70–80°C.

11. Titrate with 0.05 N potassium permanganate, taking as an end-point the first color that persists 15 to 30 seconds. If, as occasionally happens, the precipitate is colored red by the methyl red, and so colors the solution to be titrated with permanganate, this color does not interfere with the end-point, as it is quickly oxidized. The indicator is not present in sufficient amount to cause any appreciable difference in the titration. The usual precautions should be taken of standardizing the permanganate each day with standardized oxalate. 1 cc. of 0.05 N $KMnO_4$ = 0.001 gm. or 1 mg. of calcium.

EXPERIMENTAL RESULTS.

In the experiments here given no effort was made to obtain the highest degree of accuracy; they represent that obtainable in any physiological laboratory by an analyst of moderate experience. In any series of determinations reported no results have been omitted.

Our standard solutions of calcium were made from calcite, and checked by McCrudden's gravimetric method. A solution of $CaCl_2$, which contained 0.0830 gm. of Ca per 25 cc., was checked by McCrudden's method and gave the following results: 0.0831, 0.0832, 0.0831.

The results on theoretical solutions of calcium are given in Table I. A sample of urine, to which uric acid was added, analyzed by McCrudden's gravimetric, McCrudden's volumetric, and the authors' methods, yielded the results shown in Table II. On another sample of urine the calcium content was determined by the authors' method. To portions of it were added known amounts of calcium. The results are given in Table III. Six determinations were then made on a sample of urine by the authors' method and six determinations on the same urine by McCrudden's gravimetric method. One of these latter was unfortunately lost. The results are given in Table IV.

TABLE I.

Theoretical Solutions by Authors' Method.

Calcium taken.	Uric acid.	Ammonium persulfate.	Calcium present.	Calcium found.
gm.	*gm.*	*gm.*	*gm.*	*gm.*
0.0000	0.0	4.0	?	0.0002
0.0332	0.0	4.0	0.0334	0.0337
				0.0332
0.0332	0.2	4.0	0.0334	0.0334
				0.0339

TABLE II.

Urine Plus Uric Acid.

Urine.	Uric acid.	Method.	Calcium.
cc.	*gm.*		*gm.*
100	0.2	McCrudden's gravimetric.	0.0342
			0.0345
			0.0345
100	0.2	McCrudden's volumetric.	0.0541
			0.0472
100	0.2	Authors'.	0.0344
			0.0342
			0.0346

TABLE III.

Recovery of Added Calcium by Authors' Method.

Urine.	Calcium added.	Calcium obtained.	Added calcium recovered.
cc.	*gm.*	*gm.*	*gm.*
100	0.0000	0.0113	
		0.01165	
		0.0116	
100	0.0165	0.0272	
		0.0284	0.0162
		0.0274	

TABLE IV.

Calcium in Urine.

Urine.	McCrudden's gravimetric method.	Authors' method.
cc.	*gm.*	*gm.*
100	0.0152	0.0160
	0.0168	0.0159
	0.0176	0.0161
	0 0158	0.0158
	0 0148	0.0161
	Lost.	0.0159
Average..............	0.01604	0.01596

DISCUSSION.

The method yields correct results with theoretical solutions containing calcium and uric acid. The calcium in urine can also be determined accurately and added calcium is recovered. Added uric acid does not affect the results by our method but gives high results if not oxidized. The advantages of the method described are: that by oxidation of the urine the resulting solution filters nearly as rapidly as water; the conditions for the precipitation are put on a rational basis by bringing the solution to the proper hydrogen ion concentration, instead of adjusting the acidity empirically with sodium acetate; the removal of the uric acid by oxidation permits the rapid and convenient method of titrating the calcium oxalate with potassium permanganate, instead of conversion to calcium oxide and weighing; and it permits rapid and accurate determinations of calcium in urine without requiring platinum. The method is accurate within 1 per cent in determining 30 mg. of calcium.

CONCLUSION.

1. Calcium in the urine can be accurately determined, if the urine is oxidized with ammonium persulfate.
2. The calcium is precipitated as the oxalate at pH 4.8 to 5.2, and titrated with 0.05 N potassium permanganate.
3. The method requires less than one quarter the time necessary for gravimetric determinations.

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THE EFFECT OF HYDROGEN ION CONCENTRATION UPON THE DETERMINATION OF CALCIUM.

BY ALFRED T. SHOHL.

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

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McCrudden's (1, 2) method has come to be recognized as the best for the accurate determination of calcium. The method is to precipitate the calcium as oxalate in the presence of sufficient ammonium chloride to hold the magnesium oxalate in solution, and of sufficient acid to hold the calcium oxalate partly in solution. Sodium acetate is then added to decrease the acidity and to precipitate the rest of the calcium oxalate on the crystals already formed. Thus, large crystals are made which are easy to filter and are not contaminated with occluded magnesium or calcium phosphate.[1] The amount of acetate is selected so as to give a solution not acid enough to dissolve the calcium oxalate, nor alkaline enough, if cold, to allow calcium phosphate to precipitate. These directions are empiric and must be followed accurately, as the author cautions.[2] Just what determines the amount of acetate is not definite, for he states that in the ash of feces 15 instead of 8 cc. are required.[3]

The sodium acetate added regulates the acidity of the solution. Unfortunately, at the time McCrudden wrote his article, Sörensen's (3), Michaelis' (4), and Clark's (5) monographs on hydrogen ion concentration were not published. In 1913, Hildebrand (6) of the Bureau of Standards showed the necessity of a systematic investigation of analytical methods with regard to the hydrogen ion concentration. Such information is not as yet available for many types of analysis. Except that of Kramer and Tisdall (7) there is none for calcium. We have, therefore, critically examined McCrudden's method in relation to hydrogen ion concentration.

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[1] McCrudden (1), p. 99.

[2] McCrudden (1), p. 100.

[3] McCrudden (2), p. 198.

Theory Underlying the Precipitation of Calcium as Oxalate.

The determination of calcium in the presence of magnesium and phosphate depends primarily upon the solubility product of the various precipitates involved; secondarily, upon the hydrogen ion concentration.[4] As a development of the last 30 years in the chemistry of solutions, the theory of ionization has been applied to the problem of precipitation of nearly insoluble substances with great success.

Solubility Product.— Stieglitz (8) and Noyes (9) have presented very carefully in their manuals of qualitative analysis the relation between ionization and precipitation. The basic principle is the mass law, which can be stated.[5]

$$\frac{(A^+) \times (B^-)}{(A\,B)} = K$$

In non-mathematical terms, when a substance is present in water an equilibrium is established between the concentration of the undissociated salt in solution and the concentration of the ions. The value, K, is a constant depending upon the nature of the particular salt. Or, when there are two ions present which form an insoluble compound, precipitation occurs until the product of the concentration of the ions divided by the concentration of undissociated salt is a constant which is the K for that salt.

A simpler relation which holds with sufficient accuracy for practical purposes is that the product of the ions is a constant. This constant is known as the "solubility product."

$$(A^+) \times (B^-) = K$$

Obviously, we decrease the amount of either ion present by increasing the other ion. If we double A^+ we halve B^-. Hence, adding more A^+ causes more and more complete precipitation of *B*⁻. Thus, one adds an excess of oxalate ions, so that the calcium is more completely precipitated. The value of the solubility constant is very important for it determines which salt will precipitate. The salt having the smaller solubility product will precipitate and the other will remain in solution. If all the factors are known a quantitative expression can be calculated rigorously from the mass law. The solubility product determines whether calcium will be precipitated as a phosphate or carbonate or oxalate in solutions containing these acids. The solubility products of the various salts to be considered are given in Table I.

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[4] The question of the problem of occlusion and the conditions for obtaining large crystals have been discussed by McCrudden (1), p. 99.

[5] Parentheses about a symbol mean concentration. Thus, H^+ means hydrogen ion; (H^+) means hydrogen ion concentration.

Effect of Hydrogen Ion Concentrations.—Acids on dissociation always give hydrogen ions, H^+. The extent of the acidity depends upon the concentration of the hydrogen ions, (H^+) (10). The effect of hydrogen ions in precipitation can best be discussed under three heads: (*a*) the effect on basic salts, (*b*) the change in the ionization constant of the acid radicals, and (*c*) the suppression of ionization of weak acids.

The effect of acid on basic salts is to depress the ionization of the hydroxyl ions; for substances in solution are related to the ionization of water. Water itself gives H^+ ions and also OH^- or basic ions. The relation of the ions is expressed by the mass law:

$$\frac{(H^+) \times (OH^-)}{(H_2O)} = 10^{-14}$$

When one adds H^+ ions to water one depresses the ionization of the hydroxyl ions since the product of these ions is a constant. Thus, in the case of magnesium hydroxide, by adding acid one decreases $(\overline{OH})$ to such an extent that in acid solution its solubility product is never reached and hence no magnesium hydroxide can precipitate.

By adding acid one changes the ionization constant of the acid. Thus, in the case of the tri-basic phosphate the addition of H^+ ions gives the following reaction:

$$\bar{P}\bar{O}\bar{_4} + H^+ = \bar{\bar{H}}\bar{P}O_4 + H^+ = H_2\bar{P}O_4$$

The tri-basic salt can only exist in alkaline solution and as one increases the hydrogen ion concentration the di-basic and mono-basic salt must be formed. Thus, in an acid system there is no calcium phosphate but only the more soluble di-basic and mono-basic calcium phosphates. The solution, if quite acid, about pH 3.0, will have the same effect on calcium oxalate.

$$H^+ + (\bar{C}_2\bar{O}_4) \overset{\longrightarrow}{\rightleftharpoons} (H\bar{C}_2O_4)$$

By adding acid to a solution containing ions of a weak acid one converts the highly ionized salt into a slightly ionized acid, according to the equation:

$$2H^+ + (\bar{C}_2\bar{O}_4) \overset{\longrightarrow}{\rightleftharpoons} H_2C_2O_4$$

Thus one removes oxalate ions from the solution by adding acid. If the ionization is repressed below the solubility product no precipitate is formed.

Each of these factors: the effect on basic salts, the change in the ionization constant of the acid, and the suppression of ionization, influence the precipitation. Therefore, we shall consider each of the salts that may be formed in the course of the analysis of calcium in the presence of magnesium and phosphates, in an acid solution of pH 4.0 to 6.2.

Calcium Oxalate and Magnesium Oxalate.—These salts are precipitated in the presence of an excess of oxalate ions. The question is whether there is any danger of precipitating magnesium with the calcium. This has been discussed in part by Kramer and Tisdall (7). The solubility product of these salts is sufficiently different so that Gooch (11) recommends that even in the presence of ten times the amount of magnesium it is not necessary to carry out double precipitation. In acid solution this difference in solubility product is even more marked.

Acid added to a solution of calcium and magnesium oxalates favors the precipitation of the calcium in two ways. It forms the acid salts which are more soluble. The acid salt of calcium oxalate, at this acidity (pH 4.0 to 6.2) is not formed in sufficient amount to have any appreciable effect on the solubility. No study of the magnesium salts has been made, but from general evidence it is concluded that this would be more readily affected by acids and hence more soluble.

Second, the addition of acid suppresses ionization, in this case from about 40 to about 30 per cent. The diminished ionization affects the calcium oxalate much less than the magnesium oxalate as the former has a much smaller solubility product. Any acidity not great enough to form acid calcium oxalate will be a factor in preventing the formation of magnesium oxalate and in favoring the precipitation of calcium oxalate.

Magnesium Hydroxide and Magnesium Ammonium Phosphate.—Both salts are extremely insoluble but since they do not occur in acid solutions they do not precipitate. Hildebrand (6) says that the former precipitates at pH 7.4 to 8.0 and that the latter first appears at pH 6.6.

Mono-, Di-, and Tri-Basic Calcium Phosphate.—The salts of calcium form a very difficult problem since as found by Cameron (12) and his collaborators and many others, these salts vary in their composition according to the source, are decomposed by water, and take months to come to equilibrium.

For orientation we attempted to find the pH of saturated solutions of the mono-, di-, and tri-basic salts of calcium phosphate. These salts (Baker's analyzed) were washed with distilled water. The acidity of the supernatant liquids, determined by the colorimetric method (5), became roughly constant at room temperature in 24 hours and gave the following values:

$CaH_4(PO_4)_2$ = pH 5.0
$CaH PO_4$ = pH 7.4
$Ca_3(PO_4)_2$ = pH 6.8

We found that no calcium solution more acid than pH 4.0 which contained phosphates yielded a precipitate after boiling, but Patten and Mains (13) report a precipitate at pH 2.3 at 26°. But the more acid the point at which these salts precipitate, the greater is the proportion of phosphate to calcium. And, as one can see by glancing at Table I, the more acid the salt the greater is the solubility product. Therefore, at pH 4.0 to 6.4 there is no danger of any calcium being present as phosphates. At this acidity the calcium phosphates have a solubility product more than a million times as great as calcium oxalate. Breazeale (14) remarks it is quite safe to precipitate calcium phosphate quantitatively as calcium oxalate by adding oxalic acid. Therefore, there is no danger of the precipitation of calcium acid phosphate if the precipitation of calcium oxalate is carried out at the proper acidity.

TABLE I.

*Data on Solubilities.**

Salt.	Per liter.	Mols per liter.	Solubility product.
	gm.		
CaC_2O_4	0.0055	0.000044	1.9×10^{-9}
$Mg(OH)_2$	0.009	0.00015	3.5×10^{-12}
$MgNH_4PO_4$	0.05	0.0068	3.0×10^{-7}
MgC_2O_4	0.302	0.0027	4.8×10^{-5}
$Ca_3(PO_4)_2$	0.01	0.00033	2.8×10^{-18}
$CaHPO_4$	0.2	0.00147	2.0×10^{-6}
$CaH_4(PO_4)_2$	18.0	0.77	4.0×10^{-1}

* The figures are compiled from Landolt-Börnstein, Physikalisch-chemische Tabellen; A. Seidell, The solubilities of inorganic substances; The Chemische Kalender, 1914—Dictionary of Solubilities, etc.

EXPERIMENTAL APPLICATION OF THE THEORY.

It is important to verify, first, the most acid limit of acidity; second, the least acid limit; and third, the best method of obtaining the desired acidity.

Most Acid Limit.—The most acid limit is the point at which calcium oxalate begins to be converted into the more soluble acid calcium oxalate. McCrudden has determined the amount of sodium acetate, which, under the conditions of his procedure will prevent the solution of calcium oxalate.[6] We determined colorimetrically the hydrogen ion concentration of solutions precipitated according to his directions. In all solutions more acid than pH 4.4, which contain less than 6 cc. of sodium acetate, the results are low. Further experiments in Table II show that correct determinations are made at pH 4.0, which is, therefore, the most acid limit for the determination of calcium oxalate.

The Least Acid Limit.—The least acid limit is the point at which magnesium ammonium phosphate and magnesium hydroxide precipitate. According to Hildebrand this is pH 6.6 to 7.6. To fix this point experimentally we carried out the precipitation

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[6] McCrudden (1), p. 90.

McCrudden's fear that calcium phosphate would precipitate unless there were between 6 and 10 cc. of sodium acetate is groundless. To test this point experimentally we made up a solution containing 2 cc. of 10 per cent calcium chloride, 2 cc. of 10 per cent magnesium sulfate, and 2 cc. of 10 per cent sodium acid phosphate. These we precipitated according to McCrudden's directions: 10 cc. of 0.5 N hydrochloric acid, 10 cc. of 25 per cent oxalic acid, 10 cc. of 3 per cent ammonium oxalate, cooled and to them added varying amounts of 20 per cent sodium acetate solution. The results are shown in Table II. Thus, even 50 cc. of acetate are not an excess and give correct results.

We found, by experiment, that it makes no difference whether one regulates the acidity by acetic acid and sodium acetate mixtures or more conveniently and quite as accurately by adding ammonia to the acid phosphates present. We have, therefore, developed a method based upon this conclusion, the details of which are given in the following paper.

Limits of Acidity.—From the above discussion it is clear that the limits of acidity for the precipitation of calcium by oxalic acid in the presence of magnesium and phosphates are determined. The most acid limit is pH 4.0. If the solution is more acid than this the calcium oxalate is partly converted into acid calcium oxalate which is so soluble that it is not quantitatively precipitated. The least acid limit is pH 5.6. If the solution is less acid appreciable amounts of magnesium ammonium phosphate precipitate and contaminate the calcium oxalate.

SUMMARY.

The hydrogen ion concentration is an important factor in making a calcium determination. If the solution is more acid than pH 4.0 calcium oxalate is dissolved. If the solution is less acid than pH 5.6, magnesium ammonium phosphate is precipitated.

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of normal rabbits under the same conditions. The retention of the pneumococci by the endothelial cells is thought to result from the action of "endothelial opsonins" which, in turn, play an important rôle in the rapid disappearance of bacteria from the blood stream following artificial bacteremia. Tenbroeck (5) found that virulent hog-cholera bacilli were clumped and disappeared from the circulation immediately after being injected into the circulation .of normal as well as of immunized rabbits. Hopkins and Parker (6) have reported that virulent streptococci first rapidly leave the circulation of susceptible animals and then return as the infection advances. These investigators concluded, however, that the streptococci were carried back into the circulation from infected tissues, and that they were dealing with a bacteremia instead of a true septicemia. This conclusion was reached because there was no evidence of the organisms having acquired properties which made permanent residence within the circulating blood possible. More recently Delrez and Govaerts (7) have published observations which seem to show that the blood platelets perform a general "antixenique" function, i.e., these elements are very active in the removal of all kinds of foreign matter from the circulation, and possibly also in the destruction of bacteria by the process of phagocytosis and intracellular digestion. They also conclude that a genuine agglutination of bacteria does not occur in the circulation of normal animals—the phenomenon observed by one of us not being true agglutination. Meyer, Neilson and Fensier (9) are inclined to believe that in vivo agglutination does not occur, and state that the disappearance of bacteria from the blood stream is probably not due to agglutination, but to the action of the blood platelets or to a dispersion phenomenon of two colloids.

We are not prepared at present to offer an opinion on all the reports which we have mentioned, since we have not had the opportunity of studying them This paper concerns the one point which we have studied, namely: the relation of the blood platelets to the agglutination of bacteria in the blood stream of animals having an artificial bacteremia, and the part which these elements of the blood play in the abrupt disappearance of bacteria from the circulation.

The platelet theory has as its basis the fact that when certain bacterial suspensions are injected into the circulation of rabbits (or other animals) the injected bacteria and the platelets collect, within a few seconds time, into more or less compact clumps (Fig. 1, Plate

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THE RELATION OF BLOOD PLATELETS TO THE IN VIV AGGLUTINATION OF BACTERIA AND THEIR DISAPPEARANCE FROM THE BLOOD STREAM.*

By CARROLL G. BULL, M.D., and CLARA M. McKEE.

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Plate X.

In the course of a study (1) of the mechanism by which the intravenous administration of immune serum causes an abrupt disappearance of the organisms from the blood stream of rabbits having a pneumococcic septicemia or bacteremia, it was observed that the pneumococci collected into clumps—were agglutinated—immediately after the serum was injected. It was further observed that the bacterial clumps rapidly left the circulation and collected in the capillaries of the internal organs—liver, spleen, lungs, etc. Similar observations (2) were made with other bacteria—dysentery bacilli, typhoid bacilli, staphylococci, etc. Virulent pneumococci were not agglutinated when injected into the circulation of normal rabbits. The conclusion was reached that there was a definite relation between the agglutination of bacteria in the blood stream and the resistance of the animal to septicemic infections, i.e., the greater the resistance of the animal, the more extensive the agglutination, and the more rapid the disappearance of the bacteria from the circulation. This was held to be true in the case of both natural and artificially acquired immunity, active or passive.

Since the publication of the above observations and conclusions other investigators have reported observations which tend to show that our deductions were too comprehensive, exceptions to the general conclusion being pointed out. Some have even questioned the occurrence of in vivo agglutination of bacteria. Thus Kitagawa (3) reports that an inagglutinable strain of pneumococci rapidly disappeared from the circulation of immune rabbits. Manwaring and Coe (4) found that pneumococci adhere to the endothelial cells of the hepatic capillaries of immune rabbits, while they are not retained by the liver instance a serum was obtained which caused the platelets to disappear rapidly from the circulation of rabbits without affecting the leucocytes or erythrocytes.

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* From the Department of Immunology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.

IMMUNIZED ANIMALS.

Active acquired immunity.—The first series of experiments deals with actively immunized rabbits. A number of rabbits were immunized with each of the following organisms: (*a*) pneumococci, (*b*) Flexner dysentery bacilli, (*c*) typhoid bacilli, and (*d*) *Staphylococcus aureus*. The details of the individual tests are given in the following representative protocols.

Experiment 1 A: A rabbit, weight 2,150 grams, had been given four intravenous injections of killed Type 1 pneumococci. Its serum agglutinated the homologous organisms in a dilution of 1–25. A platelet count gave 375,000 per c.mm. Three c.c. of antiplatelet serum were injected into the ear vein. Two hours later the platelets had fallen to 6,000* per c.mm., and no platelets could be found in a stained specimen of blood. 80,000 million pneumococci were injected into the ear vein, and specimens of blood were taken from the heart for culture and microscopic examination as indicated in Protocol A

PROTOCOL A.

Agglutination of pneumococci in a "deplateletized" immune rabbit.

Time after pneumococci were injected.	Specimen of heart blood.	Colonies per c.c.	Bacterial clumps.	Platelets.
0' 5"*.......	1st	7,860,000	many	none
0' 40".......	2d	2,000,000	fewer	"
1' 5".......	3d	1,200,000	"	"
3' 15".......	4th	65,000	none	"
5' 55".......	5th	20,000	"	"
10' 30".......	6th	5,000	"	"

Experiment 1 B: The rabbit used in this test weighed 2,000 grams, and had been immunized with killed cultures of Type 1 pneumococci; its serum agglutinated the homologous organisms in a dilution of 1–50. A platelet count gave 350,000 per c.mm. of blood. 80,000

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* Since it is not possible to distinguish with certainty a blood platelet from particles of other materials in the counting chamber, such low platelet counts mean that there were not more and probably fewer platelets present than the figures indicate.

* '—minutes, "—seconds.

X) and that the clumps soon leave the circulating blood. Th phenomenon can easily be observed by injecting a suspension Flexner dysentery bacilli into the ear vein of a normal rabbit, or suspension of pneumococci into the ear vein of an immune rabb and taking specimens of blood from the heart from 30 seconds to minutes after the injection is made. The part of the theory whi concerns the destruction of the bacteria by the platelets is purel hypothetical.

This association of the platelets with the clumps of bacteria, or of the bacteria with the clumps of platelets, was observed in our first work on the mechanism of the abrupt disappearance of the bacteria from the circulation of resistant animals, but it was not considered to be essential to the phenomenon of in vivo agglutination, because platelets were not always attached to or mixed with the bacterial clumps (Fig. 2, Plate X), particularly in the case of immunized animals. We have attempted to answer three questions, namely: (1) Are platelets essential to the collection into masses (agglutination) of bacteria in immunized animals? (2) in normal animals? and (3) do the platelets play an essential rôle in the abrupt disappearance of bacteria from the circulation of animals in which an artificial bacteremia is produced? The general plan of study was to make identical tests in normal rabbits and in rabbits whose blood platelets had been destroyed or removed from the circulation.

EXPERIMENTAL.

General technique.—The technique of the experiments was in part the same as that previously described (1). From 2 to 5 c.c. of bacterial suspensions, of suitable densities, were injected into the ear veins of rabbits, and specimens of blood for microscopic observation and culturing were taken from the heart at intervals varying from 5 seconds to 2 hours after the injections were made. When passively immunized animals were being tested the immune serum was given intravenously a few minutes before the bacteria were injected. Platelet counts were made by Wright's method, which proved to be satisfactory for our purpose. The animals were " deplateletized " by the intravenous administration of an anti-platelet serum which was prepared by giving guinea pigs three or four intraperitoneal injections of the washed platelets from 50 to 60 c.c. of rabbit blood. The guinea pigs were bled from 7 to 10 days after the last injection, and in every

Passive acquired immunity.—The experiments just described were repeated in rabbits passively immunized to the different bacteria. The bacteria were agglutinated and disappeared from the circulation of the rabbits whose platelets had been destroyed just as quickly and completely as they did in the controls. Experiments 2 A and 2 B with typhoid bacilli will serve to illustrate the results obtained with the passively immunized animals.

Experiment 2 A: A rabbit weighing 1,800 grams was given 3 c.c. of antiplatelet serum. Two hours later a platelet count gave 7,000 per c.mm., and no platelets could be found in stained blood films. 10 c.c. of antityphoid serum was injected into the ear vein and two minutes later 70,000 million typhoid bacilli were injected into the other ear vein. Specimens of blood were taken from the heart for culture and films, as indicated in Protocol C.

Protocol C.

Agglutination of typhoid bacilli in a passively immune "deplateletized" rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Colonies per c.c.	Bacterial clumps.	Platelets.
0′ 5″	1st	20,640,000	many	none
0′ 55″	2d	6,500,000	fewer	"
2′ 15″	3d	2,006,000	"	"
4′ 15″	4th	500,000	"	"
9′ 5″	5th	60,000	none	"

Experiment 2 B: A rabbit, weight 1,850 grams, platelet count 450,000 per c.mm., received 10 c.c. of antityphoid serum in the vein and one minute later 70,000 million typhoid bacilli. Protocol D gives the details of the cultures and films of the blood specimens.

Throughout the experiments on immunized animals it was noticed that the first specimen taken after the bacteria were injected contained the greatest number of bacterial clumps without platelets. In some instances, particularly with pneumococci and dysentery bacilli, a great majority of the clumps had no platelets associated with them. The subsequent specimens contained more and more clumps of bacteria mixed with platelets. This observation leads one to believe that the bacteria are agglutinated before the platelets become attached to them. This point will be referred to again in connection with the experiments on normal rabbits.

PROTOCOL D.

Agglutination of typhoid bacilli in a passively immune " normal " rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Colonies per c.c.	Bacterial clumps.	Platelets.
0′ 5″.........	1st	18,500,000	many[*]	numerous
0′ 30″.........	2d	3,500,000	fewer	"
1′ 50″.........	3d		"	"
3′ 50″.........	4th	2,800,000	"	"
6′ 0″.........	5th	750,000	"	"
9′ 50″.........	6th	100,000	"	"

NORMAL ANIMALS.

A series of experiments was made with normal rabbits. The general technique was similar to that just described in connection with the experiments on immunized rabbits. Two rabbits of equal size were used in each test, one being deplateletized and the other not. In some instances, particularly in the experiments with *B. typhosus,* the titer of the natural agglutinins was determined and rabbits having equal titers were chosen for the individual tests. Three species of bacteria were used, namely: *B. typhosus, B. dysenteriae* (Flexner), and *Staphylococcus pyogenes aureus.* The details of the experiments are given in the following type protocols.

B. dysenteriae (Flexner).—A large number of tests were made with this organism. Since the results were regularly similar, one detailed experiment (Experiment 3) will suffice for the purpose of illustration.

Experiment 3: A platelet count was made on each of three rabbits. The number of platelets per c.mm. were, respectively, 245,000, 260,000, and 395,000. The rabbit (Protocol G) which had the highest platelet count was given 2 c.c. of antiplatelet serum in the ear vein, and 45 minutes later the count was 7,000. The rabbits were now tested for in vivo agglutination and the rate of disappearance of the bacilli from the blood stream. Each rabbit received an intravenous injection of 75,000 million dysentery bacilli, and blood specimens were taken from the heart as indicated in the respective protocols.

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[*] In the first specimen of blood there were many clumps of bacilli which were free of platelets, in the second specimen only a few and in the other specimens all of the clumps were attached to clumps of platelets.

Protocol E.

Agglutination of dysentery bacilli in a normal rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 5″	1st	+	0	+++
0′ 30″	2d	+++	0	+
0′ 50″	3d	++	++	+
0′ 10″	4th	0	+	+
1′ 30″	5th	0	+	+-
1′ 50″	6th	0	+	0

Protocol F.

Agglutination of dysentery bacilli in a normal rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 10″	1st	+	0	++
0′ 50″	2d	++	+	+
1′ 30″	3d	0	++	+
2′ 5″	4th	0	+	+-
2′ 40″	5th	0	+	0
3′ 30″	6th	0	+	0

Protocol G.

Agglutination of dysentery bacilli in a normal "deplateletized" rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 10″	1st	++	0	+
0′ 38″	2d	+++	0	+
1′ 12″	3d	+	0	+
1′ 54″	4th	+	0	+
2′ 36″	5th	+	0	0
3′ 39″	6th	0	0	0

Protocols E, F, and G present two points of interest. First, the absence of the platelets did not perceptibly influence the agglutination of the bacilli (Fig. 7, Plate X) or their disappearance from the blood stream. Second, the first and second specimens of blood from one of the rabbits (Protocol E) whose platelets had not been removed and the first specimen from the other (Protocol B) contained only clumps of bacilli with which no platelets were mixed. The following specimen in each case contained clumps of bacilli, both with and without an admixture of platelets, while in the later specimens there were only mixed clumps. This observation indicates that here again the bacteria are first agglutinated and then come in contact with clumps of platelets.

B. typhosus.—Since the sera of normal rabbits show considerable variations with respect to agglutination of typhoid bacilli, the serum of each rabbit was titrated in vitro, before the in vivo tests were made. The sera were heated at 56° C. for thirty minutes and the titers determined by the macroscopic method. The titers varied from a 1–2 to a 1–16 dilution of serum. The individual tests were made on rabbits having equal agglutinating titers. Experiment 4 illustrates the results.

Experiment 4: Three rabbits were used in this experiment. Two had agglutinating titers of 1–4, the other 1–16. One of the rabbits with a 1–4 agglutinating titer (Protocol I) and the one with the 1–16 titer (Protocol J) were given antiplatelet serum. The other rabbit (Protocol H) was used as a control. Each rabbit was given an intravenous injection of 75,000 million typhoid bacilli, and specimens of blood were taken from the heart at short intervals and examined microscopically to determine the extent of the agglutination of the bacilli and the rate of their disappearance from the circulation. The observations are recorded in Protocols H, I, and J.

PROTOCOL H.

Agglutination of typhoid bacilli in a normal rabbit (control).

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 7″........	1st	0	0	+++
0′ 47″........	2d	+	+	++
2′ 21″........	3d	0	++	+
4′ 18″........	4th	0	+++	+
7′ 22″........	5th	0	++	+
10′ 1″........	6th	0	+	+
17′ 13″........	7th	0	0	+
20′ 3″........	8th	0	0	+

PROTOCOL I.

Agglutination of typhoid bacilli in a normal " deplateletized " rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 11″........	1st	++	0	++
1′ 0″........	2d	+++	0	+
2′ 4″........	3d	++	0	+
4′ 0″........	4th	+	0	+
7′ 30″........	5tb	+	0	+
10′ 15″........	6th	0	0	+
15′ 21″........	7th	0	0	+
20′ 19″........	8th	0	0	0

PROTOCOL J.

Agglutination of typhoid bacilli in a normal " deplateletized " rabbit.

Time after bacilli were injected.	Specimen of heart blood.	Clumps of bacilli.	Clumps of bacilli and platelets.	Free bacilli.
0′ 7″.........	1st	++	0	++
0′ 23″.........	2d	+++	0	+
1′ 5″.........	3d	++	0	+
1′ 35″.........	4th	++	0	+
2′ 30″.........	5th	+	0	+
3′ 31″.........	6th	+	0	+
4′ 10″.........	7th	0	0	0
5′ 2″.........	8th	0	0	0

It is shown by Experiment 4 that typhoid bacilli agglutinate (Fig. 8, Plate X) in the blood stream of normal rabbits, and also that the bacilli rapidly leave the circulation. Both the agglutination and the disappearance of the bacilli from the circulation seem to be directly related to the potency of the natural agglutinins rather than to the presence of the blood platelets. The agglutinating titers of the sera of the rabbits used in Protocols II and I were the same, 1–4. The bacilli left the circulation with about equal rapidity. The difference was in favor of the deplateletized rabbit (Protocol I), no bacilli being found in the films of the 20-minute specimen of blood, while they were found in the corresponding specimen from the control rabbit. Agglutination occurred in both instances. In the normal rabbit (Protocol H) platelets were mixed with all of the clumps, with the exception of the second specimen, which contained some platelet-free clumps of bacilli, while there were no platelets in any of the specimens from the deplateletized rabbit. In both cases the unagglutinated bacilli were the last to disappear. In Protocol J no bacilli were found in the specimens taken 4 minutes and 10 seconds after the bacteria were injected. This rabbit had been deplateletized, but the serum had a high agglutinating titer, 1–16.

Other similar tests were made with rabbits whose sera had very low agglutinating titers. The bacilli were only slightly agglutinated and remained in the circulation a comparatively long time, no appreciable reduction in the number of organisms occurring within ten minutes after the injections were made. The results were the same, however, in the normal and the deplateletized rabbits.

Staphylococcus pyogenes aureus.—This organism was selected because the sera of normal rabbits agglutinate it only slightly, or not at all. A large number of rabbits were tested for natural agglutinins, and none of the sera agglutinated the cocci within two hours at 37° C. If the tests were kept at 37° C. for two hours and then allowed to remain at room temperature for 18 hours, agglutination occurred in some instances in 1–4 and 1–8 dilutions of serum. However, when a rabbit was tested several times, at short intervals, the results were very irregular, and the presence or absence of natural agglutinins was disregarded. The procedure was the same as with the other bacteria, i.e., the rabbits were paired, one being given antiplatelet serum and the other not. The blood specimens were both plated and examined microscopically. A number of tests were made, from 14,000 million to 80,000 million cocci being injected, but since the results were uniformly similar only two experiments will be given in detail.

Experiment 5 A: Two rabbits of equal weight were selected. One had a platelet count of 375,000, the other 400,000. The latter (Pro-

PROTOCOL K.

Disappearance of staphylococci from the blood stream of a normal rabbit.

Time after cocci were injected.	Specimen of heart blood.	Colonies per c.c.	Clumps of platelets and cocci.	Free cocci.
0′ 15″........	1st	innumerable (0.005 cc.)	few cocci on clumps of platelets	many
15′ 15″........	2d	31,000	no cocci	none
36′ 0″........	3d	15,000	" "	"
56′ 0″........	4th	10,000	" "	"

tocol L) was given 3 c.c. of antiplatelet serum. Both rabbits were given 15,000 million staphylococci in the ear vein, and four specimens of blood were taken from the heart, at intervals of about 15 minutes. See Protocols K and L.

PROTOCOL L.

Disappearance of staphylococci from the blood stream of a "deplateletised" normal rabbit.

Time after cocci were injected.	Specimen of heart blood.	Colonies per c.c.	Clumps of platelets and cocci.	Free cocci.
0′ 10″........	1st	innumerable (0.005 cc.)	no platelets	many
15′ 15″........	2d	30,000	" "	none
31′ 5″........	3d	11,000	" "	"
65′ 35″........	4th	2,400	" "	"

PROTOCOL M.

Disappearance of staphylococci from the blood stream of a normal rabbit.

Time after cocci were injected.	Specimen of heart blood.	Colonies per c.c.	Clumps of platelets and cocci.	Free cocci.
0′ 10″.........	1st	innumerable (0.005 cc.)	few cocci on clumps of platelets	many
0′ 34″.........	2d	innumerable	many cocci on clumps of platelets	"
0′ 48″.........	3d	15,860,000	many cocci on clumps of platelets	fewer
2′ 13″.........	4th	1,060,000	very few on clumps of platelets	"
2′ 57″.........	5th	950,000	very few on clumps of platelets	"
3′ 51″.........	6th	600,000	very few on clumps of platelets	"
5′ 10″.........	7th	540,000	very few on clumps of platelets	"
7′ 0″.........	8th	350,000	very few on clumps of platelets	"

Experiment 5 B: Two rabbits, each weighing 2,000 grams, had platelet counts of 425,000 and 372,000 respectively. The rabbit having the lowest platelet count was given 3 c.c. of antiplatelet serum. Both rabbits were given 14,000 million staphylococci in the ear vein and eight specimens of blood were taken from the heart during the succeeding 7 minutes. The details are given in Protocols M and N.

PROTOCOL N.

Disappearance of staphylococci from the blood stream of a "deplateletised" normal rabbit.

Time after cocci were injected.	Specimen of heart blood.	Colonies per c.c.	Clumps of platelets and cocci.	Free cocci.
0′ 11″.........	1st	innumerable (0.005 cc.)	no platelets	many
0′ 31″.........	2d	innumerable	" "	"
0′ 49″.........	3d	7,460,000	" "	"
1′ 38″.........	4th	2,300,000	" "	fewer
2′ 25″.........	5th	550,000	" "	"
3′ 30″.........	6th	140,000	" "	"
5′ 17″.........	7th	90,000	" "	"
6′ 3″.........	8th	40,000	" "	none

Experiments 5 *A* and 5 *B* show that the blood platelets do not play an essential rôle in the disappearance of staphylococci from the blood stream of normal rabbits, and also that in vivo agglutination does not occur, except possibly to a slight degree in some instances. In the first experiment (5 *A*) the blood specimens were taken from 10 seconds to about one hour after the cocci were injected into the ear vein, and it is seen that comparatively few organisms are in the blood at the expiration of 15 minutes, and that the number is only slightly reduced after this time. There was no appreciable difference in the rate of disappearance in the normal and the deplateletized rabbit, the slight difference being in favor of the rabbit whose platelets had been destroyed. Experiment 5 *B* covers only the first phase of the disappearance curve. Here again cocci left the circulation of the deplateletized rabbit somewhat more rapidly than that of the normal rabbit. Our conclusion is that the platelets do not materially influence the disappearance curve, the discrepancies in the number of organisms in the corresponding blood specimens being due to variations in other respects in the individual rabbits and to imperfections of the tech-

nique. At least, the absence of the platelets does not retard the disappearance of the bacteria from the circulating blood. The collection of the cocci on clumps of platelets must be, therefore, an incidental phenomenon.

DISCUSSION.

The observations recorded above show quite definitely that bacteria are agglutinated immediately after being injected into the blood stream of immunized animals. The reaction is completed within a few minutes, and the clumped bacteria disappear from the circulating blood. In normal rabbits, i.e., rabbits whose platelets have not been destroyed, platelets are frequently mixed with the bacterial clumps. It is evident, however, that the platelets are not essential to the formation of the bacterial clumps since, even with the normal number of platelets present, many clumps have no platelets associated with them. It is indicated, moreover, that in the formation of the mixed clumps (platelets and bacteria) the bacteria are first agglutinated and then pick up the platelets, because the earlier specimens of blood contain many more platelet-free clumps of bacteria than the later specimens. The experiments with immunized rabbits whose blood was practically free of platelets show conclusively that the blood platelets are in no way essential to the phenomenon of in vivo agglutination, and the abrupt disappearance of the bacteria from the blood stream. With normal rabbits the results are variable and depend upon the kind of bacteria used. Flexner dysentery bacilli regularly behave in normal rabbits as pneumococci do in immunized rabbits, i.e., they are actively agglutinated and leave the circulation independently of any influence of the platelets. Varying results were obtained with typhoid bacilli, particularly with regard to agglutination. If the serum contained few or no agglutinins for this organism, there was little or no clumping of the bacilli in deplateletized rabbits, but they collected on clumps of platelets in the control rabbits. If the serum contained more agglutinins, the bacilli were agglutinated and rapidly left the blood stream in the absence of platelets. The clumping and disappearance of the bacilli from the circulation were directly related to the agglutinin content of the serum, and were independent of the number of platelets present. *Staphylococcus aureus* did not agglutinate in the normal rabbits whose platelets had been destroyed, but collected, to a greater or less extent, on clumps of platelets in rabbits having the normal number of platelets. But even in this case the cocci left the circulating blood just as quickly in the deplateletized as in the control rabbits. It is concluded, therefore, that neither the agglutination of bacteria within the blood stream nor their disappearance from it can be ascribed to a function of the platelets.

It now seems possible to define clearly the sequence of events which occurs when bacterial suspensions are injected into the blood stream. If the animal possesses an appreciable amount of agglutinins the bacteria are immediately agglutinated and rapidly leave the blood stream, collecting in the capillary systems of the liver, spleen, lungs, etc. As the clumps of bacteria form they may become associated with clumps of platelets, before leaving the blood stream, but the platelets are not essential either to the agglutination or to the disappearance of the bacteria from the blood stream, since they both occur when the blood contains no platelets. If the bacteria are not agglutinated, but are so affected as to make them more " adhesive " (opsonized?) they become attached to clumps of platelets and then collect in the capillary systems. Here, again, the platelet phase is merely incidental, for when platelets are not present the bacteria adhere directly to the capillary endothelium, leaving the circulating blood as quickly as when the platelets are present. If the bacteria are neither agglutinated nor opsonized, as is the case with virulent pneumococci in normal rabbits, they do not leave the blood stream abruptly, but continue to circulate in the blood and produce a septicemia without a reduction in the number of organisms having occurred. The " adhesive " effect mentioned above is considered to be a preagglutination effect, i.e., if the bacteria were more intensely affected agglutination would result. It is due to such an action on the part of the plasma that staphylococci rapidly leave the blood stream of normal rabbits and collect in the viscera, and it offers an explanation of the observations of Kitagawa (3) on inagglutinable pneumococci and of Manwaring and Coe (4) on the collection of pneumococci in the hepatic capillaries, as the result of treating the organisms with an immune serum which was diluted beyond its agglutinin titer. The plasma of normal rabbits does not affect virulent pneumococci even to this extent, and consequently these organisms remain in the blood stream.

SUMMARY AND CONCLUSIONS.

1. When bacterial suspensions are injected into the circulation of immunized rabbits, or of normal rabbits having natural agglutinins,

the bacteria are immediately agglutinated and rapidly leave the blood stream. The blood platelets, when present, become attached to a majority of the clumps of bacteria, but both the agglutination and the disappearance of the bacteria from the circulation, of either normal or immunized animals, are just as complete and rapid, when the platelets are absent.

2. Suspensions of *Staphylococcus aureus* are not agglutinated, when injected into the circulation of normal rabbits, but the bulk of the organisms rapidly leave the blood stream. When platelets are present they collect into clumps to which many of the cocci become attached before leaving the circulation, but the cocci leave the circulation with equal rapidity when no platelets are present. In this case the bacteria leave the blood stream because the plasma so alters them that they adhere to the endothelial cells of the capillary systems.

3. Virulent pneumococci remain in the circulation of normal rabbits, because they are neither agglutinated nor opsonized.

4. Depending upon the antibody content of the blood, one of three things occurs when artificial bacteremias are produced, viz.: (1) the bacteria are neither agglutinated nor opsonized, and continue to circulate in the blood stream, (2) the bacteria are agglutinated, or are both agglutinated and opsonized, and rapidly leave the circulating blood and collect in the capillary systems of the liver, spleen, lungs, etc., or (3) the bacteria are not agglutinated, but are opsonized and rapidly leave the circulating blood and likewise collect in the internal organs.

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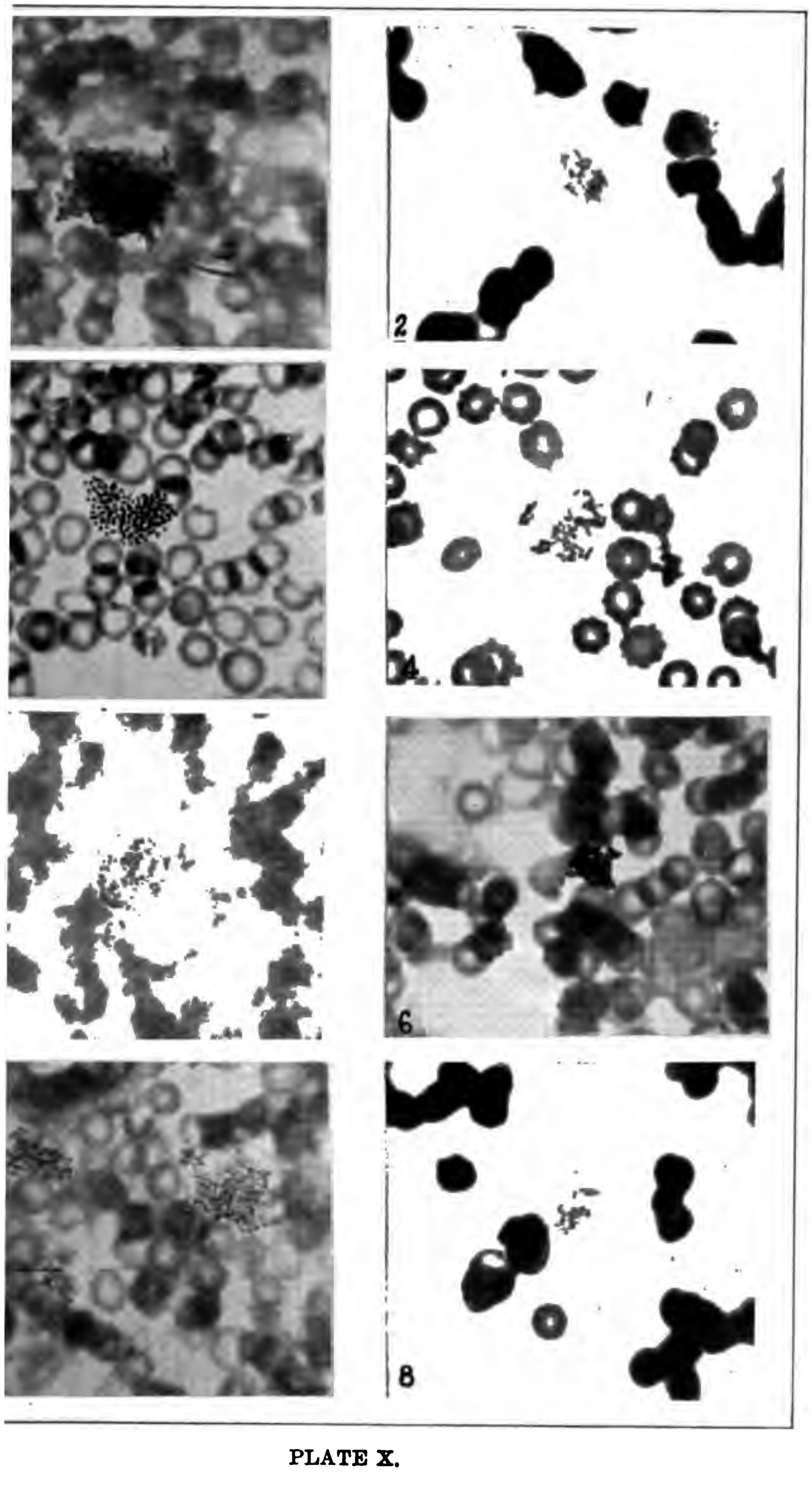

PLATE X.

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ON THE PRODUCTION OF HEMOLYSINS AND HEMAGGLUTININS IN THE DOMESTIC FOWL.*

BY ROSCOE R. HYDE, PH.D., AND CLENNIE E. BAILEY.

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The production of immune hemolytic sera was first demonstrated by Bordet (1) in 1898. He injected rabbit blood into the guinea pig and made the far-reaching observation that the serum from the treated animal became powerfully lytic for rabbit corpuscles. Subsequent observations on different species of animals and with many different kinds of red corpuscles have shown that Bordet's discovery holds as a general principle in immunological reactions, i.e., the serum of an animal that has been treated with the red corpuscles of another species becomes powerfully lytic for these cells. The acquired lytic property is due to an increase in the thermostabile amboceptor or sensitizer content of the serum. It is equally true, on the other hand, that all animal species do not react equally to immunization with the corpuscles of a particular species, and that a given species does not react in the same manner to different kinds of corpuscles. For example, the cat and the dog respond very poorly, compared with the rabbit, to intraperitoneal injections of sheep corpuscles. The rabbit yields a most potent sensitizer for sheep corpuscles but produces a less potent serum for human corpuscles. Very potent hemagglutinating and hemolytic serum is obtained from the rabbit by intravenous injections of fowl cells, while with ox cells one obtains a serum of high hemolytic titer, but the agglutinating titer is practically nil. Further examples might be cited to show that it is not possible to predict the result of immunizing a given species with a particular kind of cell and that each case must be determined by trial.

The present paper deals with the production of hemolysins and hemagglutinins in the domestic fowl. Citron (2) specifically mentions the domestic fowl as one of the species most suitable for the production of hemolytic sera. In his " Immunity " (Garbat translation, 1912, page 131) the following statement is found: " As far as the technique for obtaining immune-hemolysins is concerned, the rules which hold for every process of immunization are naturally to be followed here also. It is not possible, however, to immunize every kind of animal against every type of blood corpuscle. Rabbits, goats, horses, and chickens are the ones which are best adapted to supply hemolytic sera. An animal produces a better hemolysin the remoter its relationship to the animal from which the erythrocytes for injection are taken.'' No evidence is given to justify the inclusion of the domestic fowl among the species best adapted to produce hemolytic sera, and, so far as is known to me, none exists. In spite of this fact Citron's statement is still taken at its face value. Thus, Guyer and Smith (3) have recently made use of it in support of their contention of having produced in the chicken a serum lytic for the eye lens of the rabbit, with which results of great biological significance were obtained. The serum of chickens which had been treated with rabbit lens was injected into the circulation of pregnant rabbits. Some of the young of these rabbits had an eye defect which was passed on to succeeding generations. It was contended that the eye defect was in all probability due to the cytolytic action of the chicken serum, since chickens were known to be good cytolysin (hemolysin) producers.

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* From the Department of Immunology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.

Before taking up the experimental work it is necessary to point out that chicken serum is distinctly peculiar with respect to the reactivation of its hemolytic action. As a rule, sera containing either native or acquired hemolysins can readily be reactivated with fresh serum from the same or other species. Heated or aged chicken serum is an exception to this rule. Reactivation does not occur when the corpuscles, sensitizer, and complement are mixed in the usual way. It is necessary to mix and incubate the sensitizer and complement, before the corpuscles are added, if hemolysis is to be effected (4).

EXPERIMENTAL

Domestic fowls were treated over varying periods of time with the blood corpuscles of the following species: pigeon, sheep, guinea pig, ox, and rabbit. The agglutinating and hemolytic titers of the serum were determined both before and during the course of the treatment. A record of the titration, the kind of corpuscles used, the routes of injection, etc., is given in connection with each individual case.

General technique. Fresh corpuscles were used both for the injections and titrations. The blood was collected in a solution of sodium citrate, the corpuscles sedimented by centrifugalization, washed four times in large volumes of salt solution, and finally enough salt solution was added to make the volume the same as that of the whole blood. Any further dilution is indicated in the individual protocols. The chicken serum was obtained by collecting the blood from the wing vein and centrifugalizing it after clotting had occurred.

The hemolytic power of the fresh serum was determined without the addition of foreign serum as complement. This will be spoken of as the " active " hemolytic titer. The test was made by putting varying quantities of undiluted serum and of $\frac{1}{10}$ or $\frac{1}{100}$ dilutions in a series of serological tubes, bringing the volume up to 0.9 c.c. with salt solution and then adding 0.1 c.c. of a $\frac{1}{4}$ dilution of corpuscles. The tubes were thoroughly shaken and put in the water bath (37° C.) for one hour. Readings were made at the end of the incubation period. The degree of hemolysis was noted and indicated in the records by numbers, 4 signifying complete, 1- a trace of hemolysis, and 3, 2 and 1 intermediate degrees. The agglutinating power of the sera was determined in a similar manner, after inactivation at 55° C. for 30 minutes.

During the first part of the work we were unable to reactivate the hemolytic property of the serum after it had been heated at 55° C. for 30 minutes. A number of fresh sera were used as complement, and our results were uniformly negative. It was found later that the natural hemolysins for rabbit cells could be reactivated after inactivation at 55° C. with either fresh guinea pig or fresh chicken serum. This point will be considered more fully in connection with the protocols in which it was employed.

Experiment 1 (Rabbit corpuscles). A large Plymouth Rock hen, whose serum had an " active " hemolytic titer of $\frac{1}{35}$, was given first, 15 subcutaneous, and then 2 intravenous injections of rabbit corpuscles during the course of three months. The injections were made at from 2 to 7 day intervals, a total of 62.5 c.c. of corpuscles being given. The second intravenous injection (2 c.c. of corpuscular suspension) caused a severe reaction, simulating an anaphylactic shock. The hen was found dead one week later. The serum was tested for both hemolysins and hemagglutinins at six different times during the course of the treatment. The hemolytic titer was not raised. In fact, on two occasions it was lower than the original titer. The agglutinins showed a slight increase on the last test, the titer being $\frac{1}{500}$, while it had previously been $\frac{1}{400}$.

Experiment 2 (Rabbit corpuscles). A large male fowl was given

3 c.c. of rabbit cells intravenously. No perceptible reaction followed this injection. Three days later a similar injection was followed immediately by labored breathing and loss of power of locomotion. Two days after the second injection 4 c.c. of corpuscles were given intravenously. Within less than two minutes the rooster staggered and fell to the floor. Breathing became increasingly difficult and finally ceased in desperate efforts to take in air. Death ensued five minutes after the injection was made.

Experiment 3 (Sheep corpuscles). A large Plymouth Rock hen was given 11 subcutaneous and 2 intravenous injections of sheep corpuscles. The injections were made at from 3 to 8 day intervals during the course of two and a half months, a total of 43 c.c. of cell suspension being given. The last two injections were given intravenously. The intravenous injection of 3 c.c. of cells was followed by a severe reaction from which the hen soon recovered. The second intravenous injection caused an immediate anaphylactic-like reaction and death.

The serum of this chicken was tested for hemolytic power at different times during the course of the injections. The titer was only $1/_{10}$ one week after the last subcutaneous injection was made.

Experiment 4 (Sheep corpuscles). The "active" hemolytic titer of the serum of the hen used in this experiment was $1/_{10}$ and the agglutinating titer $1/_{33}$. She was given 11 intraperitoneal injections of sheep corpuscles during six weeks time. An average of 15 c.c. of cell suspension was given at each injection. The serum was retested six times after the injections were begun. Four weeks after the first injection, four injections having been given, the hemolytic titer was $1/_{50}$ and the agglutinin titer $1/_{100}$. Both titers subsequently became weaker, and at the last test were only $1/_{10}$.

Further immunization experiments, similar to those given above, were carried out in which ox, guinea pig, and pigeon erythrocytes were used as antigens. Subcutaneous, intraperitoneal and intravenous injections were made during long periods of time. The results were not materially different from those already given. There was never more than a slight increase in the hemolytic power of the sera, and the agglutinating titers did not increase more than two- or four-fold. These increases came after the third or fourth injection, and were followed by a return to the original titers.

It may be argued that the method which we have used to determine the hemolytic power of the sera of the treated animals was faulty, since, in the dilution of the sera the complement was soon reduced to the minimum requirement of this factor, and that hemolysis failed to occur in higher dilutions because of a deficiency of complement and not because the hemolytic sensitizer was not increased as a result of immunization. That such was not the case finds support in the well-known fact that when the hemolytic amboceptor is increased less complement is required to effect a given amount of hemolysis. Thus, according to Noguchi, the amount of complement necessary to hemolyse one unit of red cells is $\frac{1}{3}$, $\frac{1}{5}$ and $\frac{1}{10}$ of a unit when, 4, 10 and 20 units respectively of amboceptor are used. Hence a four-fold increase in the sensitizer content of a fresh serum should raise the titer three-fold; a ten-fold increase, five-fold; and a twenty-fold increase, ten-fold. Hence, the conclusion that the hemolysin content of the sera of the fowls was not greatly increased as a result of the extensive treatment with foreign red cells would seem to be justified.

When the work had progressed to this point it was found that the natural hemolysin in chicken serum for rabbit corpuscles could be reactivated with either fresh guinea pig or chicken serum, after being heated at 55° C. for 30 minutes. Consequently two other fowls were given a series of injections of rabbit cells and the sera titrated both before and after inactivation. The titrations with foreign complement were carried out as follows: The serum from normal or immunized fowls was heated in 5 c.c. quantities at 55° C. for 30 minutes. Graded quantities of the whole serum, or of suitable dilutions of the serum, were put into a series of tubes, and salt solution was added to bring the volume in each tube up to 0.8 c.c. A subhemolytic quantity of fresh chicken or guinea pig serum in a volume of 0.1 c.c. was added to each tube. The tubes were shaken and put in the water bath (37° C.) for 30 minutes. 0.1 c.c. of a $\frac{1}{4}$ dilution of rabbit erythrocytes was now put in each tube. The tubes were put in the water bath again for one hour. By this method the hemolytic power of heated normal chicken serum can be completely restored, while if the corpuscles, heated serum and fresh serum were put into the tubes at the same time, or if the heated serum and corpuscles were mixed before the fresh serum was added, no hemolysis occurred. The heated serum and the fresh serum must be mixed and incubated before the corpuscles are added.

Experiment 5 (Rabbit corpuscles). The serum of the fowl used in this experiment had an " active " hemolytic titer of $\frac{1}{25}$ for rabbit erythrocytes, and the heated serum (55° C. for 30 minutes) agglutinated the cells in a dilution of $1/250$. The inactivated serum plus a subhemolytic dose (0.02 c.c.) of fresh chicken serum gave a hemolytic titer of $1/25$. The fowl was then given four intraperitoneal injections of 3.5 c.c. each of rabbit corpuscles, the injections being given at two-day intervals. Six days after the last injection the serum gave an active titer of $1/50$. The titer of the inactivated serum, using chicken serum as the source of complement, was between $1/25$ and $1/50$. When fresh guinea pig serum was used as complement the titer was $1/5$. The hemagglutinins were not materially increased. It is evident that treatment had little influence upon the sensitizer and agglutinin content of the serum.

Experiment 6 (Rabbit corpuscles). A large Plymouth Rock rooster was given eight injections of rabbit erythrocytes during the course of eleven days, a total of 25 c.c. of cell suspension being given. The cells were injected both intravenously and into the peritoneum. The first treatment consisted of an intravenous injection of 4 c.c. of cell suspension. Two days later 2 c.c. of cells were injected into the peritoneal cavity. Three further treatments were given at three-day intervals. These consisted of an intraperitoneal injection of from 2 to 4 c.c. of cells and, three hours later, an intravenous injection of an equal amount. This method of treatment was adopted because of the severe and often fatal anaphylactic-like shocks that usually follow the intravenous injection of cells in fowls which have had one or more previous injections. This particular fowl survived the treatment, but the desensitizing action of the intraperitoneal injections was not entirely effective, since rather severe reactions always followed the intravenous injections.

Before treatment was begun the serum of this fowl had a hemolytic titer of $1/33$ and an agglutinating titer of $1/250$. Six days after the last treatment the titers were $1/500$ and $1/1000$ respectively. Subhemolytic doses (0.02 c.c.) of fresh chicken serum were used for complement in the hemolytic reactions. It is seen, therefore, that in this instance the potency of the serum was definitely raised as a result of the treatment. A similar result was not obtained in any other case.

RABBIT ERYTHROCYTES RENDERED NON-ANTIGENIC BY CHICKEN SERUM.

The failure of the domestic fowl to respond energetically to immunization with foreign erythrocytes raises a question of general interest. Is the failure due to an absence of the proper receptors or affinities in the tissues of this animal, or may it not be due to the fact that the body fluids render the foreign cells non-antigenic before they can reach or stimulate the proper tissues of the host? The energetic way in which chicken serum acts upon the red blood cells of the rabbit in vitro suggested the latter as a possibility and experiments were planned to test this point.

Experiment 7. Five guinea pigs were given respectively three intraperitoneal injections, at 4-day intervals, of the following mixtures: Guinea pig 1, 1 c.c. of rabbit corpuscles in 8 c.c. of fresh chicken serum; Guinea pig 2, 1 c.c. of rabbit corpuscles in 8 c.c. of distilled water; Guinea pig 3, 1 c.c. of rabbit corpuscles in 8 c.c. of salt solution; Guinea pig 4, 8 c.c. of chicken serum; Guinea pig 5, 1 c.c. of sheep corpuscles in 8 c.c. of fresh rabbit serum. The mixtures were allowed to stand at room temperature for twelve hours, and were then put at 37° C. for 3 hours before they were injected. This procedure was repeated three times. Seven days after the last injection the guinea pigs were bled and their sera were tested for hemolysins in the usual way. The results were as follows: Guinea pig 1, no hemolysins for rabbit cells; Guinea pig 2, hemolytic titer for rabbit cells, 1–1250; Guinea pig 3, hemolytic titer for rabbit cells, 1–1250; Guinea pig 4, no hemolysins for rabbit cells; Guinea pig 5, hemolytic titer for sheep cells, 1–2000. Experiment 7 was repeated four times with practically the same results. It is seen that rabbit red blood cells hemolysed with fresh chicken serum no longer cause the formation of specific hemolysins in the guinea pig, while water hemolysis does not alter the antigenic properties of the cells. It is further seen that hemolysis by serum does not necessarily destroy the antigenic properties of the hemolysed cells, for sheep cells hemolysed with fresh rabbit serum still engendered the formation of specific antibodies when injected into guinea pigs.

DISCUSSION AND SUMMARY.

Normal chicken serum lyses and agglutinates the erythrocytes of many other species. The lytic property depends upon two factors, one thermostabile, the other thermolabile. When the serum becomes inactive as a result of exposure to heat or through aging, the lytic property is not restored by the addition of fresh serum from other species to the usual hemolytic tests. Reactivation can be effected, however, by mixing and incubating the heated and fresh serum before the corpuscles are added.

The foregoing work shows that chickens do not respond to treatment with foreign red blood cells as most other species do. In only one instance was there a material increase in the lytic power of the serum. This peculiarity on the part of the chicken is of interest in connection with the theories of the origin and nature of antibodies, and a knowledge of the mechanism here involved may aid in understanding failures to stimulate antibody formation under other conditions.

If antibodies are cast-off receptors, the tissues of the chicken should be well supplied with receptors for rabbit corpuscles, because we find numerous free receptors in the blood stream. Antigenic stimulation should give rise to increased production of receptors, and the serum should become more potent as the result of an accumulation of free receptors or antibodies. It is evident that the theory is wrong or that the injected cells or their constituents do not reach the cell receptors and stimulate the formation of an excess of receptors and thus new antibodies. Since it has been shown that fresh chicken serum acts upon rabbit blood cells in vitro in such a way as to render the cells non-antigenic for guinea pigs, it seems probable that a similar thing occurs in vivo and that the foreign cells are rendered nonantigenic, before the tissues of the host have been sufficiently stimulated. A similar condition may obtain in other instances where animals do not respond to immunization.

In the light of our observations on the production of hemolysins in the chicken, it seems improbable that Guyer and Smith (3) produced in this species a serum lytic for the eye lens of the rabbit. At least, the conclusion that cytolysins must have been formed in the chickens treated with the rabbit lenses because of the readiness with which this species produces cytolytic sera, is not tenable.

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SEGREGATION AND RECOMBINATION OF THE GENES FOR TINGED, BLOOD, BUFF, AND CORAL IN DROSOPHILA MELANOGASTER.

ROSCOE R. HYDE.

From the Department of Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.

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In a previous paper I reported the origin of blood and tinged, two sex-linked eye mutants in Drosophila. Both mutants gave complete linkage with white, eosin and cherry and consequently formed with the red of the wild type a sextuple system of multiple allelomorphs. Safir and Lancefield later reported buff and coral, two other members of this system, as shown by their linkage to white. T. H. Morgan has kindly supplied me with stocks of buff and coral and this paper records the combinations made with these two stocks to my stocks of blood and tinged. The results of these crosses bear out the expectation that, since blood and tinged are allelomorphic to white and buff and coral are allelomorphic to white, the mutants buff, coral, tinged and blood should show allelomorphism to each other.

The evidence from such a system is significant as it bears on the nature of the change that takes place in the chromosomes of mutant stocks. If the different mutants are a result of losses of materials that lie at different levels on the sex-chromosome, then the wild type eye should result in the daughters on crossing any two of these mutants, since the daughter receives two sex-chromosomes and each would restore the missing allelomorph of the other. As a matter of fact the daughters from all combinations are com-

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pounds, with eyes intermediate in color between the parent types. If the genes for these different eye colors are allocated at different levels on the sex chromosome there should appear among the grandsons eyes of the wild type as a result of the phenomenon of crossing over. The evidence presented from these combinations is consistant with that obtained from other tests in showing that this does not occur.

The nomenclature employed is that used in other publications for the members of this system,—white w, tinged w^t, buff w^{bu}, eosin w^e, cherry w^c, coral w^{co}, blood w^b, red W.

The female is expressed by the formula XX, the male XO, consequently the formula for a tinged eyed female is w^tw^tXX. a tinged eye male w^tXO.

The following tables give the results of the combinations made between the different members of this allelomorphic series. The genetic composition of the parents (P), the children (F_1), and grandchildren (F_2) are expressed in terms of the nomenclature stated above. The numbers indicate the number of flies realized from the different combinations.

1. *Linkage of Buff and Tinged*

(a) Buff ♀ by Tinged ♂.

P.	$w^{bu}w^{bu}XX$.	w^tXO
F_1.	$w^{bu}w^tXX$	$w^{bu}XO$
(1).	52	58
(2).	200	185
(3).	406	301

F_2	$w^{bu}w^{bu}XX$ $w^{bu}w^tXX$	$w^{bu}XO$ w^tXO.
1	135	112
1a.............	155	110
2	84	56
2a.............	58	28
3	129	136
3a.............	83	65
4	136	118
4a.............	45	53
5	91	70
5a.............	130	93
6	75	115
6a.............	85	96
7	23	30
7a.............	95	108
Total.......	1324	1190

The buff females and the tinged-buff compounds are practically inseparable. The two classes of males can be separated into buff and tinged males but are here classified together. The wild red eyed form did not appear in the F_1 or F_2 generation. The F_2 males and females show a slight variation in color although we were unable to separate the two on a color basis.

(b) Buff ♂ by Tinged ♀

P.	$w^{bu}XO$.	w^tw^tXX
F_1	$w^tw^{bu}XX$	w^tXO.
(1)	30	31
(2)	256	200
(3)	94	85

The tinged males and the tinged-buff compound females of the F_1 are inseparable on a color basis as one might expect since the two colors are separated by such a small margin.

F_2	w^tw^tXX	$w^tw^{bu}XX$	w^tXO	$w^{bu}XO$.
1	72		48	
2	127		103	
3	113		65	
4	242		186	
5	68		47	
6	112		82	
Total	734		531	

The F_2 males can be separated with difficulty.

2. *Linkage of Buff and Blood.*

(a) Buff ♀ by Blood ♂.

P.	$w^{bu}w^{bu}XX$.	w^bXO
F_1	$w^{bu}w^bXX$	$w^{bu}XO$
	202	198

F_1 Females compounds uniform deep red—males like buff.

F_2	$w^{bu}w^{bu}XX$	$w^{bu}w^bXX$	$w^{bu}XO$	w^bXO
1	51	72	50	69
2	45	46	49	37
3	130	148	117	119
4	158	152	168	139
Total	384	418	384	364

Males and females in the F_2 are easily separated into the different classes.

(b) Buff ♂ by Blood ♀.

P. $w^{bu}XO$. $w^{b}w^{b}XX$

F^1 $w^{bu}w^{b}XX$ $w^{b}XO$

117 104

F_1 Males typical of blood eyes—light when young—turn **very dark with** age. Females lighter than blood and do not turn so dark with age.

F_2	wbwbuXX	wbwbXX	wbuXO	wbXO
1....................	79	83	60	58
2....................	66	79	45	54
3....................	139	182	147	112
4....................	79	67	74	77
Total............	363	411	326	301

3. *Linkage of Coral and Tinged.*

(a) Coral ♀ by Tinged ♂.

P. $w^{co}w^{co}XX$. $w^{t}XO$

F_1	wcowtXX 27		$w^{co}XO$ 22	
F_1 Flies	Lighter than coral stock		Same color as coral stock	
F_2	$w^{co}w^{co}XX$	$w^{co}wtXX$	$w^{co}XO$	$w^{t}XO$
1	79	94	79	84
1a..................	78	70	75	56
1b..................	46	40	33	29
2	74	78	65	47
2a..................	57	62	62	58
3	40	43	51	54
Total............	374	387	365	328

(b) Coral ♂ by Tinged ♀.

P. $w^{co}XO$. w^tw^tXX

F_1	$w^tw^{co}XX$ 44		w^tXO 36	
F_2	w^tw^tXX	$w^tw^{co}XX$	w^tXO	$w^{co}XO$
1	31	48	33	35
1a	63	60	61	60
1b	5	7	4	8
2	68	54	74	52
2a	68	68	53	61
2b	24	18	9	14
Total	259	255	234	230

4. *Linkage of Coral and Blood.*

(a) Coral ♀ by Blood ♂.

P. $w^{co}w^{co}XX$. w^bXO

F_1	$w^{co}w^bXX$ 18		$w^{co}XO$ 18	
F_1 Flies	Female just a hint lighter than male.		As these flies become older the sexes are indistinguishable.	
F_2	$w^{co}w^{co}XX$	$w^{co}w^bXX$	$w^{co}XO$	w^bXO
1	116		103	
1a	159		135	
2	49		81	
2a	180		178	
Total	945		497	

The two classes of males can be separated when young. Females cannot be separated with certainty. They show a variable range as is true of blood which overlaps the coral.

(b) Coral ♂ by Blood ♀.

P. $w^{co}XO$. w^bw^bXX

F_1	$w^bw^{cu}XX$ 18	w^bXO 17
F_1 Flies	Females and males same color; dark as males. A mere hint that females are lighter.	
F_2	w^bw^bXX $w^bw^{cu}XX$	w^bXO $w^{cu}XO$
1	103	116
1a	143	107
1b	21	27
2	162	146
2a	126	122
2b	82	70
Total	637	588

5. *Linkage of Coral and Buff.*

(a) Coral ♀ by Buff ♂.

P. $w^{co}w^{co}XX$. $w^{bu}XO$

F_1	$w^{cu}w^{bu}XX$ 31		$w^{cu}XO$ 29	
F_1 Flies	Much lighter than coral		Same color as stock coral.	
F_2	$w^{cu}w^{cu}XX$	$w^{cu}w^{bu}XX$	$w^{cu}X0$	$w^{bu}XO$
1	57	40	49	44
1a	50	56	45	40
Total	107	96	94	84

(b) Coral ♂ by Buff ♀.

P. $w^{cu}XO$. $w^{bu}w^{bu}XX$

F₁	$w^{bu}w^{cu}XX$ 44		$w^{bu}XO$ 37	
F₁ Flies	Lighter than buff Lighter than coral Lighter than blood?		Same color as buff stock.	
F₂	$w^{bu}w^{bu}XX$	$w^{bu}w^{cu}XX$	$w^{bu}XO$	$w^{cu}XO$
1	57	41	46	45
1a	253	233	239	277
1b	45	25	33	29
2	198	203	166	175
2a	20	19	29	20
Total	573	521	513	446

Compound ♀ much lighter than ♂.

6. *Linkage of Tinged and Blood.*

(a) Tinged ♀ by Blood ♂.

P. $w^{t}w^{t}XX$. $w^{b}XO$

F₁	$w^{t}w^{b}XX$ 33		$w^{t}XO$ 29	
F₁ Flies	All lighter than blood, but darker than tinged.		Males like tinged stock.	
F₂	$w^{t}w^{t}XX$	$w^{t}w^{b}XX$	$w^{b}XO$	$w^{t}XO$
1	74	98	71	78
1a	109	105	104	97
2	37	36	21	32
2a	57	52	46	63
2b	29	22	26	28
Total	306	313	268	298

(b) Tinged ♂ by Blood ♀.

P. w^tXO . w^bw^bXX

F_1	w^tw^bXX 18		w^bXO 17	
F_1 Flies	Females much lighter than males.		Typical of blood stock Sexes easily separated on color basis.	
F_2	w^bw^bXX	w^bw^tXX	w^bXO	w^tXO
1	52	55	41	45
1a	68	68	47	61
1b	9	9	5	4
2	35	31	42	47
2a	164	160	112	136
2b	45	45	26	33
Total	373	368	273	326

7. *Linkage of Coral to Eosin.*

(a) Eosin ♀ and Coral ♂.
P. $w^e w^e XX$. $w^{co} XO$

F_1	$w^e w^{co} XX$ 108 200		$w^e XO$ 94 200	
F_1 Flies	Indistinguishable from coral compound does not seem to modify.		Typical of eosin; slight color change with age.	
F_2	$w^e w^e XX$	$w^e w^{co} XX$	$w^e XO$	$w^{co} XO$
9a	60	55	60	57
	40	54	71	47
11a	30	30	24	35
	60	68	65	70
11b	15	10	14	16
	62	60	59	48
F_2 Flies	Like Eosin ♀ ♀	Slightly darker than ♀ but not as dark as males.	Like eosin ♂.	Much like blood when old but not as dark

(b) Eosin ♂ by Coral ♀.

P. w^eXO . $w^{cu}w^{cu}XO$

F_1	$w^ew^{cu}XX$ 125	$w^{cu}XO$ 115	
F_1 Flies	The eye colors are hard to distinguish. The females seem to be a little lighter than the males; darker when old.		
F_2	$w^{cu}w^{cu}XX$ $w^{cu}w^eXX$	w^eX	$w^{cu}X$
1	166	70	90
1a...............	59	36	40
1b...............	107	17	34
2	196	98	97
3	87	40	50
3a...............	80	31	41
Total.........	695	292	352

8. *Summary.*

The genes for buff and coral known to be allelomorphic to white have here been tested with tinged and blood, two other genes allelmorphic to white. The expectation is that since both are allelomorphic to white, they will be allelomorphic to each other. The results of the different combinations made verify the expectations. Sufficient evidence has accumulated to show that these are members of the same allelomorphic series. The fact that the red eyed fly does not appear in the F_1 or F_2 bears out the assumption that the different members of this multiple allelomorph series are but different expressions of the same material particle and that they occupy identical loci on the sex-chromosome.

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BEHAVIOR OF THE GENE FOR THE MUTANT CURVED OF DROSOPHILA MELANOGASTER IN CROSSES INVOLVING GENES IN THE SAME AND OTHER CHROMOSOMES.

ROSCOE R. HYDE.

From the Department of Immunology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore.

Contents.

I. Behavior of the curved winged mutant with normal wild stock.
   1. Curved ♀ by wild ♂. Chromosome I.

II. Behavior of curved with bent. Chromosome IV.
   1. Curved ♀ by bent ♂.

III. Behavior of curved with spineless. Chromosome III.
   1. Curved ♀ by spineless ♂.
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   4. Black, purple curved ♂ by curved ♀.
   5. Crosses involving black, purple, vestigial and curved.

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A wing mutation in Drosophila melanogaster which has proved to be identical with curved, a mutant found by Bridges in rudimentary stock, appeared in my wild cultures in the latter part of November, 1914. The culture was one of a large number of wild stocks that were taken during the fall of 1913

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in Indiana. Twelve curved of both sexes appeared simultaneously in the culture bottle. The mutant has been kept in pure culture since that time. A number of crosses with mutants known to belong to different chromosome groups are here recorded.

I. Behavior of the curved winged mutant with normal wild stock. Chromosome I.

1. Curved ♀ by wild ♂.

A curved wing female bred to the normal long wing male produced 18 sons and 13 daughters,—all with wings typical of wild stock. These when inbred gave in the F_2 generation 107 curved and 348 wild type flies. Table I. Both sexes are affected. This result demonstrates curved to be a recessive, non sex-linked mutant giving a Mendelian ratio of approximately 3 to 1. It is evident that the gene for curved is not carried by chromosome I, since it does not show sex-linkage.

Table I. F_2 generation from curved ♀ by wild ♂.

Number	Normal ♂♂	Normal ♀♀	Curved ♂♂	Curved ♀♀
1	48	46	8	11
1a	52	32	21	18
1b	46	55	13	13
1c	24	45	13	10
Total.	170	178	55	52

II. Behavior of curved with bent. Chromosome IV.

1. Curved ♀ by bent ♂.

Bent is a wing mutant first described by Muller who demonstrated that its gene belongs to Group IV. The curved female by bent male gave all normal flies of the wild type in the F_1 generation. These inbred produced in F_2 the different classes given in table II.

Table II. F_2 generation from curved ♀ by bent ♂.

Wild type ♂♂	Wild type ♀♀	Curved ♂♂	Curved ♀♀	Bent ♂♂	Bent ♀♀
66	90	37	29	33	31

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Table I. F_2 generation from curved ♀ by wild ♂.

Number	Normal ♂♂	Normal ♀♀	Curved ♂♂	Curved ♀♀
1	48	46	8	11
1a	52	32	21	18
1b	46	55	13	13
1c	24	45	13	10
Total.	170	178	55	52

II. Behavior of curved with bent. Chromosome IV.

1. Curved ♀ by bent ♂.

Bent is a wing mutant first described by Muller who demonstrated that its gene belongs to Group IV. The curved female by bent male gave all normal flies of the wild type in the F_1 generation. These inbred produced in F_2 the different classes given in table II.

Table II. F_2 generation from curved ♀ by bent ♂.

Wild type ♂♂	Wild type ♀♀	Curved ♂♂	Curved ♀♀	Bent ♂♂	Bent ♀♀
66	90	37	29	33	31

This cross should give a ratio of 9:3:3:1 if the gene for curved is in a chromosome other than IV. Bent is a poor character with which to contrast curved since both characters involve the wings and the double recessive cannot with certainty be distinguished. A few forms appeared, however, which I took to be curved bent. Counting the double recessive with curved and bent a ratio of 9 to 7 should result. The table gives approximately this ratio and a tentative conclusion reached that the gene for curved is borne by a chromosome which gives free assortment with IV.

III. Behavior of curved with spineless. Chromosome III.

1. Curved ♀ by spineless ♂.

Spineless, a mutant characterized by the absence of bristles on the thorax, was first described by Bridges. Its gene belongs to Chromosome III. All ♀ children from the curved by spineless ♂ were characteristic of the wild type. These when inbred in pairs gave in the F_2 generation the different classes as shown in table III.

Table III. F_2 generation from curved ♀ by spineless ♂.

Number of Mating	Wild type	Normal wing spineless	Normal spines curved	Curved Spineless
1	75	21	22	5
2	57	14	11	4
3	57	17	19	8
4	58	18	13	8
5	89	16	22	2
6	58	15	14	4
7	61	22	13	5
8	9	2	2	0
9	39	14	8	4
10	55	1	11	1
11	42	2	12	0
12	77	24	20	10
13	63	2	14	0
14	35	9	9	4
15	82	25	24	5
16	46	13	14	6
17	78	20	18	8
18	87	25	28	8
19	49	1	24	0
20	48	11	14	5
21	60	23	14	4
22	48	18	8	4
23	40	2	13	0
24	47	17	13	1
Total......	**1360**	**332**	**360**	**96**

2. Curved ♀ by vestigial ♂.

All the F_1 generation from this combination have long wings of the wild type as is the expectation. These when inbred produced the different classes as given in tables VII and VIII.

Table VII. F_2 generation from curved ♀ by vestigial ♂.

Number	Wild type		Curved		Vestigial	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1	208	203	89	82	69	90
1a	81	97	38	40	30	32
2	154	130	74	69	62	56
2a	89	112	30	34	9	17
3	156	124	61	64	63	78
3a	128	139	61	53	36	36
4	104	88	51	39	49	48
4a	70	83	21	37	17	24
5	193	208	76	96	78	87
5a	84	105	15	19	7	9
6	40	51	22	20	16	10
6a	36	39	17	22	17	19
7	41	49	9	9	15	12
7a	52	47	16	8	17	19
8	64	78	40	34	41	39
8a	71	93	22	16	23	29
9	93	75	35	26	38	35
9a	58	64	27	24	20	27
Total..	1722	1785	704	692	607	667

3. *Back crosses between the hybrid of curved and vestigial to the parent stocks.*

The genetic relation between curved and vestigial was studied in various combinations of back crosses between the hybrids and the parent stocks. Tables VIII—XV shows the different combinations made and the classes realized.

Table VIII. Hybrid ♀ (curved ♀ by vestigial ♂) by curved ♂.

Number	Wild type		Curved	
	♂♂	♀♀	♂♂	♀♀
1	87	84	53	62
1a	116	160	101	84
2	104	125	63	74
2a	3	11	2	0
3	87	107	66	77
Total......	884		582	

Table IX. Hybrid ♂ (curved ♀ by vestigal ♂) by curved ♀.

Number	Wild type		Curved	
	♂♂	♀♀	♂♂	♀♀
1	155	141	103	98
2	83	93	61	94
Total......	472		356	

Table X. Hybrid ♀ (curved ♀ by vestigial ♂) by vestigial ♂.

Number	Wild type		Vestigial	
	♂♂	♀♀	♂♂	♀♀
1	82	92	43	58
2	55	53	54	38
3	90	91	35	33
4	45	28	29	29
Total......	536		319	

Table XI. Hybrid ♂ (curved ♀ by vestigial ♂) by vestigial ♂.

Number	Wild type		Vestigial	
	♂♂	♀♀	♂♂	♀♀
1	45	41	34	34
1a	89	87	44	48
2	26	18	19	11
2a	10	7	4	2
3	95	118	92	83
3a	72	87	37	45
Total....	695		453	

Table XII. Hybrid ♀ (vestigial ♀ by curved ♂) by vestigial ♂.

Number	Wild type		Vestigial	
	♂♂	♀♀	♂♂	♀♀
	85	101	35	31
Total......	186		66	

Table XIII. Hybrid ♂ (vestigial ♀ by curved ♂) by vestigial ♀.

Number	Wild type		Vestigial	
	♂♂	♀♀	♂♂	♀♀
	74	87	32	22
Total......	161		54	

Table VIII. Hybrid ♀ (curved ♀ by vestigial ♂) by curved ♂.

Number	Wild type		Curved	
	♂♂	♀♀	♂♂	♀♀
1	87	84	53	62
1a	116	160	101	84
2	104	125	63	74
2a	3	11	2	0
3	87	107	66	77
Total......	884		582	

Table IX. Hybrid ♂ (curved ♀ by vestigal ♂) by curved ♀.

Number	Wild type		Curved	
	♂♂	♀♀	♂♂	♀♀
1	155	141	103	98
2	83	93	61	94
Total......	472		356	

Table X. Hybrid ♀ (curved ♀ by vestigial ♂) by vestigial ♂.

Number	Wild type		Vestigial	
	♂♂	♀♀	♂♂	♀♀
1	82	92	43	58
2	55	53	54	38
3	90	91	35	33
4	45	28	29	29
Total......	536		319	

Table XVI. F_2 generation from straight black ♀ by gray curved ♂.

Number	Straight gray	Straight black	Curved gray	Curved black
1	80	39	20	0
2	114	30	36	0
3	219	104	67	0
Total......	413	173	123	0

2. Curved ♀ by black ♂.

The reciprocal cross in which the curved ♀ is mated to the black ♂ gave all wild type in the F_1 generation. These inbred gave results as given in table XVII. These results show very clearly that there is linkage between black and curved since the double recessive does not appear in F_2.

Table XVII. F_2 generation from gray curved ♀ by straight black ♂.

Number	Straight gray	Straight black	Curved gray	Curved black
1	131	41	31	0
2	139	45	31	0
3	104	58	34	0
Total......	374	144	96	0

3. Curved ♀ by black purple curved ♂.

Tables XVIII and XIX give additional data in which curved was crossed to the triple recessive black purple curved obtained from Morgan in the fall of 1918. The curved ♀ by the black purple curved ♂ gave 200 gray red eyed curved sons and 218 gray red eyed daughters. The fact that all the F_1 had curved wings is conclusive proof that Morgan's curved stock and mine are identical. The independent change affecting the germ plasm had changed identical genes. Matings were made from the F_1 with the results recorded in table XVIII.

Table XVIII. F_2 generation from curved ♀ by black, purple curved ♂.

Number	Gray red curved	Black purple curved	Black red curved	Gray purple curved
1	128	35	6	6
1a	180	35	10	7
2	199	45	10	8
2a	116	32	5	2
3	123	30	7	8
3a	119	32	1	2
4	165	43	2	5
4a	44	14	1	3
Total......	1074	276	49	42

4. Black purple curved ♀ by curved ♂.

The F_1 from the reciprocal cross to that in 3 gave 202 gray red eyed curved sons and 231 gray red eyed curved daughters. These inbred gave in F_2 the different classes in table XIX.

Table XIX. F_2 generation from black, purple, curved ♀ by curved ♂.

Number	Gray red curved	Black purple curved	Black red curved	Gray purple curved
1	248	52	6	25
2	273	59	14	11
3	313	77	11	7
4	263	59	13	6
Total......	1097	247	44	49

The fact that all the flies in the F_1 and F_2 are curved shows that the two mutants are identical. The last two tables give data on the linkage of purple and black. There are 184 cross overs in a total of 2878 a linkage of 12.8.

5. The Black, purple vestigial was crossed to a new wild stock from Arlington, Md. Over 500 sons and daughters were like the wild type. Since crossing over does not take place in the males, F_1 females whose composition was (b p v B P V) were back crossed to black purple vestigial (b p v). The

females produce non-crossover gametes (b p v) and (B P V) and crossover gametes of the composition b P V—Bpv—bpV—BPv—bPv and Bpv. The males to which they are mated produce gametes of the composition b p v. The different classes realized are given in table XX.

Table XX. Classes realized on back crossing the bpv ♂ to the hybrid ♀ of composition bpv BPV.

No.	Vestigial				Long			
	Black		Gray		Black		Gray	
	Purple	Red	Purple	Red	Purple	Red	Purple	Red
	bpv	bPv	Ppv	BPv	bpV	bPV	BpV	BPV
1	95	2	15	23	16	13	1	119
2	62	4	6	5	8	14	2	78
3	111	4	14	9	15	16	2	89
4	179	2	12	26	62	36	4	301
Totals.	447	12	47	63	101	79	9	587

VI. Summary.

The independent origin of curved, a wing mutant identical **with** the one reported by Bridges, is here given. **The data obtained** with the various crosses is in **agreement with the great mass of evidence** which shows that the chromosome conception of **inheritance offers** the only rational basis upon which such data can **be interpreted. Genes** carried by different chromosomes give free assortment **with Mendelian** ratios, those in the same chromosome show **linkage with no crossing over** in the male.

It is evident that the factor grouping of a new mutant can be determined by the failure of the double recessive to appear in the F_2 generation. Morgan has made use of black-pink flies for testing a new mutant type. Black is in the second group and pink in the third. If the new factor belongs to either of these groups it will fail to show the double recessive among the grandchildren from the cross. The sex relations **from such an experiment** will readily determine whether or not it belongs to the **sex-linked group. If it belongs** to none of these groups it is by exclusion **placed in the fourth** group.

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May 19, 1922

THE DOMESTIC FOWL AS A SOURCE OF IMMUNE HEMOLYTIC SERA

DURING the last three years we have obtained abundant evidence which refutes Citron's[1] claim that the chicken is one of the best adapted animals for the production of hemolytic sera. Citron gave no evidence to justify the inclusion of the domestic fowl among the species best adapted to produce hemolytic sera and so far as known to me, none exists. In point of fact, we find this animal one of the poorest hemolysin producers that has come within our experience.

It was known to Bordet[2], Sachs[3], Metchnikoff[4], and P. Müller[5] long before the appearance of Citron's book, that a difficulty was involved in demonstrating the sensitizer or amboceptor content of the serum of this animal[6], and Citron's unsupported claim should have been regarded with suspicion. In spite of this fact, the statement from Citron is still taken at its face value.

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[1] Citron, J., 1912, *Immunity*. Translation by A. L. Garbat.

[2] Bordet, J., 1899, "Agglutination et dissolution des globules rouges," *Ann. de l'Inst. Pasteur*, 13: 273.

[3] Sachs, Hans, 1902, *Berl. klin Wochen*, Nos. 9 and 10.

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THE USE OF THE ORIGINAL DIAGNOSTIC CULTURE FOR THE DETERMINATION OF THE VIRULENCE OF DIPHTHERIA BACILLI.

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THE USE OF THE ORIGINAL DIAGNOSTIC CULTURE FOR THE DETERMINATION OF THE VIRULENCE OF DIPHTHERIA BACILLI.*

BY LEON C. HAVENS, M.D., AND HORACE M. POWELL.

(Received for publication January 3d, 1922.)

It is frequently desirable to know whether a throat culture, which is positive on the basis of morphology, actually contains toxin-producing diphtheria bacilli. This is particularly the case when one is dealing with convalescent patients and healthy carriers. Since the virulent and avirulent types of this organism cannot be distinguished by means of their cultural and morphological characteristics, it is necessary to resort to animal tests. The animal test most commonly used consists of giving a guinea pig a subcutaneous injection of a certain quantity of a broth culture, which has been inoculated from an isolated colony of a plate made from the diagnostic culture. As a rule a control pig, which has been given antitoxin, is given the same amount of culture as the test animal. The criterion for virulence is the death of the test pig or the nature of the local lesion. The procedure entails the isolation of pure cultures and the use of two animals for each culture tested. Aside from the time and expense involved, this method is not absolutely satisfactory in that the test culture comes from only one of many colonies and when a negative result is obtained one is faced with the possibility of having selected a colony of avirulent organisms while other colonies from the same diagnostic culture contained virulent organisms. It would be a decided advantage, therefore, to have a method which would reduce the items of time and expense, be more representative of the whole diagnostic culture and, at the same time, give accurate end results.

In 1915 Zingher and Soletsky (1) introduced the intracutaneous method of making diphtheria virulence tests. This method differs from the subcutaneous method in that the cultures, after isolation, are grown on solid medium, suspended in salt solution and injected into the skin of a guinea pig. Since the reaction (when the proper

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* From the Department of Immunology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.

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THE USE OF THE ORIGINAL DIAGNOSTIC CULTURE FOR THE DETERMINATION OF THE VIRULENCE OF DIPHTHERIA BACILLI.*

BY LEON C. HAVENS, M.D., AND HORACE M. POWELL.

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It is frequently desirable to know whether a throat culture, which is positive on the basis of morphology, actually contains toxin-producing diphtheria bacilli. This is particularly the case when one is dealing with convalescent patients and healthy carriers. Since the virulent and avirulent types of this organism cannot be distinguished by means of their cultural and morphological characteristics, it is necessary to resort to animal tests. The animal test most commonly used consists of giving a guinea pig a subcutaneous injection of a certain quantity of a broth culture, which has been inoculated from an isolated colony of a plate made from the diagnostic culture. As a rule a control pig, which has been given antitoxin, is given the same amount of culture as the test animal. The criterion for virulence is the death of the test pig or the nature of the local lesion. The procedure entails the isolation of pure cultures and the use of two animals for each culture tested. Aside from the time and expense involved, this method is not absolutely satisfactory in that the test culture comes from only one of many colonies and when a negative result is obtained one is faced with the possibility of having selected a colony of avirulent organisms while other colonies from the same diagnostic culture contained virulent organisms. It would be a decided advantage, therefore, to have a method which would reduce the items of time and expense, be more representative of the whole diagnostic culture and, at the same time, give accurate end results.

In 1915 Zingher and Soletsky (1) introduced the intracutaneous method of making diphtheria virulence tests. This method differs from the subcutaneous method in that the cultures, after isolation, are grown on solid medium, suspended in salt solution and injected into the skin of a guinea pig. Since the reaction (when the proper amount of culture is injected) is limited to a local skin lesion, from 4 to 6 cultures can be tested on one animal. This procedure reduces the expenditure for animals, but it is just as time consuming and is no more representative of the whole culture than the subcutaneous method.

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* From the Department of Immunology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.

While engaged in a study of diphtheria which entailed the isolation of from 10 to 20 pure cultures daily, we received a personal communication from Dr. John N. Force, University of California, advocating the use of whole diagnostic cultures, instead of pure cultures, for making virulence tests by the intracutaneous method. We have made a series of tests with whole cultures, checking the results with pure cultures isolated from them, by both the intracutaneous* and the subcutaneous methods. A summary of the work is presented in the present paper.

EXPERIMENTAL.

The cultures.—The cultures, 509 in number, were from patients and contacts—diagnostic and release cultures—and from healthy individuals not associated with cases. The cultures were collected during the different seasons of the year in order that the contaminating organisms might be as widely varied as possible. The cultures were made on Loeffler's serum medium and incubated from 12 to 18 hours.

Preparation of the cultures for injection. After the microscopic examination, the culture was washed from the medium with 1 or 2 c.c. of sterile salt solution and transferred to a clean sterile tube and further diluted as desired. The secondary dilution was roughly proportional to the relative number of diphtheria bacilli present, more salt solution being added to the cultures which contained numerous bacilli than to those which contained only a few. The heaviest cultures were suspended in 5 or 6 c.c. of salt solution.

The injection. It is advisable to use guinea pigs weighing more than 400 grams because a larger number of tests can be made on one animal and the lesions do not spread as readily as they do in smaller animals. The hair is removed from the ventral surface by plucking and carefully shaving off the short, fine hairs which the plucking

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*Before beginning the work with whole cultures, 677 pure cultures had been tested for virulence by the intracutaneous method. More than 100 of these tests were checked by the subcutaneous method. The intracutaneous method proved to be quite satisfactory, when the proper care was used in regard to the number of bacilli injected. This was controlled by diluting the suspensions to a standard opacity, about 20 million organisms being injected.

TABLE 1.

Virulent and avirulent diphtheria bacilli from the same throat culture.

Cultures.	Number of colonies picked.	Number of colonies giving virulent cultures.	Number of colonies giving avirulent cultures.
147.........	33	32	1
153.........	8	8	0
154.........	7	7	0
155.........	12	12	0
157.........	2	2	0
158.........	20	20	0
160.........	14	13	1
161.........	18	16	2
162.........	12	12	0
164.........	13	13	0
182.........	16	16	0
185.........	14	14	0
189.........	12	11	1
190.........	2	2	0
191.........	18	18	0
204.........	3	3	0
244.........	6	6	0
Total 17.....	210	205	5

of staphylococci or streptococci which had been isolated from throat cultures were mixed with " sub-positive " numbers of the diphtheria bacilli and an intracutaneous virulence test made with the mixture. The results are summarized in Table 2.

TABLE 2.

Intracutaneous virulence tests with artificially mixed cultures.

Number of diphtheria bacilli.	Number of staphylococci.	Number of streptococci.	Result.
400,000	0	0	+
300,000	0	0	−
200,000	0	0	−
300,000	300,000	0	+
200,000	200,000	0	+
200,000	20,000,000	0	+
300,000	0	300,000	+
200,000	0	200,000	+
200,000	0	20,000,000	+

It is seen from Table 2 that 400,000 diphtheria bacilli in pure culture gave a positive test, while 200,000 and 300,000 failed to produce a characteristic lesion. However, when mixed with an equal number of staphylococci or streptococci 200,000 diphtheria bacilli were sufficient to cause a positive lesion. It is further seen that the diphtheria bacilli produce a characteristic lesion even when there are 100 times as many staphylococci or streptococci as diphtheria bacilli present. The apparent enhancement of the virulence of the diphtheria bacilli by the staphylococci or streptococci is probably due to the fact that the destruction of the bacilli is retarded, giving them a longer time in which to produce toxin. It is less probable that the cocci render the tissues of the guinea pig more susceptible to the necrotizing action of the toxin.

CONCLUSIONS.

1. The original diagnostic culture can be used advantageously for the diphtheria bacillus virulence test by the intracutaneous method.
2. The method eliminates the necessity of isolating pure cultures and makes it possible to test any desired number of cultures.
3. The results obtained by this method check with those obtained by the pure culture methods, and, moreover, it is possible to determine the virulence of cultures when pure cultures cannot be isolated.

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Cytamoeba Bacterifera in the Red Blood Cells of the Frog

R. W. HEGNER
Department of Medical Zoology, School of Hygiene and Public Health,
Johns Hopkins University

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CYTAMOEBA BACTERIFERA IN THE RED BLOOD CELLS OF THE FROG

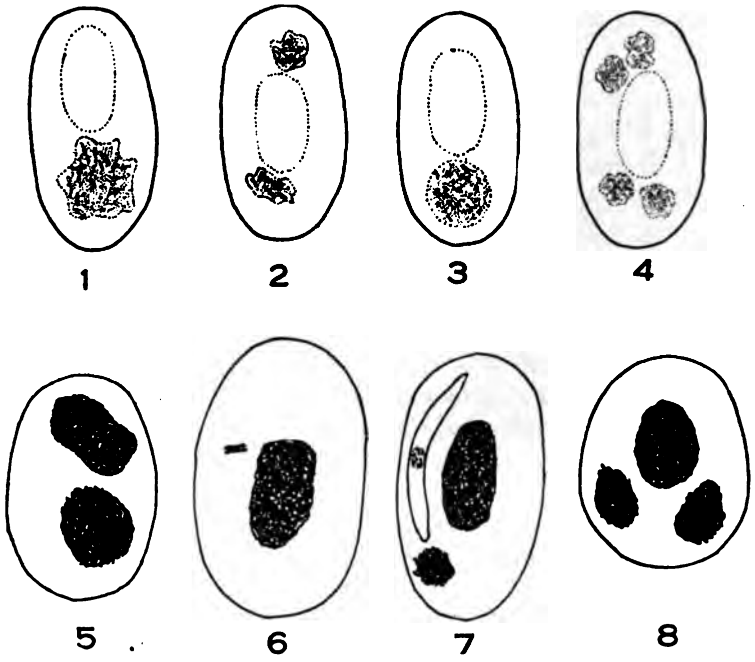
R. W. Hegner

Department of Medical Zoology, School of Hygiene and Public Health,
Johns Hopkins University

This note is intended to call to the attention of parasitologists and bacteriologists the presence in the red blood cells of frogs in this country of a most interesting organism to which the name *Cytamoeba bacterifera* was given by Labbé in 1894.

Cytamoeba bacterifera was found in the red cells in the peripheral blood of two specimens of *Rana clamitans* and five specimens of *R. catesbiana* collected at Cold Spring Harbor, Long Island, N. Y., in the summer of 1919. It was absent from the other frogs and toads examined; namely, *Rana palustris, R. sylvatica, R. pipiens, Hyla versicolor, Bufo americanus* and *Scaphiopus holbrookii.* In every case the host was also parasitized with trypanosomes, hemogregarines, and several other forms that represent either undescribed species or stages in the life cycles of parasites known only as adults. Freshly drawn blood was studied as well as material fixed and stained by Wright's method and by the Schaudinn-iron-haematoxylin method. The frogs were kept alive in the laboratory for a considerable period and their blood examined at intervals of a few days; several of them were kept in this way for three months.

The importance of studying specimens of Cytamoeba while alive and in freshly drawn blood was soon demonstrated, since movement ceases shortly after being taken from the frog's body and the appearance in stained preparations is far from normal. A typical, fully grown specimen as seen when alive and active is shown in figure 1. It measures about 7μ in diameter and occupies a position at one end of the red cell, sometimes pushing the nucleus out of its usual central position. The entire parasite is active as well as the bacilliform bodies within it. It resembles very much a trophozoite of the tertian malarial parasite, *Plasmodium vivax*, throwing out and withdrawing pseudopodia on all sides. At the same time the bacilliform bodies are moving about rapidly within, creating a flame-like appearance. After a few minutes the movements of the parasite cease and a stationary, rounded shape i assumed (Fig. 3), but the bacilliform bodies continue to move abo within, often for several hours. Finally all movement of both parasit and bacilliform bodies ceases. Many stages in the growth of the par site are present within the red blood cells of a frog at the same tim The first recognizable stages appear like minute vacuoles frequent situated at the side of the nucleus of the red cell instead of at one en as later. Only one or a few bacilliform bodies may be present at th time (Fig. 6). As growth proceeds the size of the Cytamoeba increas as well as the number of bacilliform bodies. Fully grown parasit differ with respect to the number of these bodies, some being supplie



All figures were drawn with a camera lucida × 1440.

Fig. 1. Large, living, active specimen of *Cytamoeba bacterifera* within r blood cell of bullfrog. The nucleus of the blood cell is pushed out of i normal position. The parasite has thrown out pseudopodia and is filled wi active bacilliform bodies.

Fig. 2. Red blood cell containing two living, active specimens.

Fig. 3. Red blood cell containing one living specimen that has rounded u and has ceased to throw out pseudopodia. The bacilliform bodies within a still active.

Fig. 4. Red blood cell containing four living, active specimens.

Fig. 5. Red blood cell containing a large specimen. This and figures 6, and 8 were drawn from material fixed and stained by Wright's method.

Fig. 6. Red blood cell containing a very young specimen with only tw bacilliform bodies.

Fig. 7. Red blood cell infected with both Cytamoeba and Haemogregarin

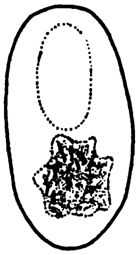
Fig. 8. Red blood cell containing two specimens of Cytamoeba.

with more than others. Blood specimens from frogs that were kept in the laboratory were examined at intervals for three months but no other stages in the life history of the Cytamoeba were found and no great differences were observed in the percentage of infection of red blood cells or in the sizes of the organisms present. For example, in a bull frog (No. 23) the percentage of infection of red blood cells ranged from 4 to 7 at different times during a period of three months. The infection in other specimens ranged from 1% up to 12%. In a few cases red blood cells contained two Cytamoebas (Figs. 2 and 8) and in one case four were present (Fig. 4). Several cells were infected with both Cytamoeba and Haemogregarina (Fig. 7). No visible difference was observed between the size and contents of the infected and noninfected red cells either in fresh blood or in the fixed and stained material such as is sometimes so conspicuous when cells are parasitized by large specimens of Haemogregarina or Karyolysus. No specimens of Cytamoeba were found free in the blood as reported by Labbé (1894) and Laveran (1899).

Specimens fixed and stained by Wright's method present an entirely erroneous appearance. The amoeboid or rounded shape of the parasite is entirely lost due probably to drying and an irregular mass of bacilliform bodies is left. These stain deeply and are quite conspicuous. The appearance of the parasite and its contained bodies is indicated in the accompanying figures (Figs. 5-8). Specimens fixed in Schaudinn's solution and stained with iron-haematoxylin are unsatisfactory. They keep their rounded shape, but do not stain well.

Several interesting questions regarding Cytamoeba remain to be solved. Is Cytamoeba an organism or only a vacuole in which bacteria live? If it is an organism is it an "adult" stage or a developmental stage of an unknown species or of a known species such as *Haemogregarina ranarum* or *Trypanosoma rotatorium*? If it is a stage of an unknown species, what stage in the life cycle does it represent and in what part of the body do the other stages occur? Are the bacilliform bodies bacteria? If so, how does the Cytamoeba become infected by them?

Kruse (1890), probably the first to figure this parasite, speaks of it not as an organism but as a space in the blood. Gabritchewsky's (1890) attention was called to it by Metchnikoff who found it in the blood of frogs infected with *Haemogregarina ranarum*. His efforts were chiefly directed toward an investigation of the "bacteria," nevertheless the "petite boule protoplasmique transparente" which contained these bacteria was considered "un être complètement, étrange à l'hématie, une espèce d'amibe, ou de larve amibiforme envahie elle-même par un microbe bactérien." Four uninfected frogs were inoculated with the spleen, liver, kidney and bone marrow of a frog con-movements of the parasite cease and a stationary, rounded shape is assumed (Fig. 3), but the bacilliform bodies continue to move about within, often for several hours. Finally all movement of both parasite and bacilliform bodies ceases. Many stages in the growth of the parasite are present within the red blood cells of a frog at the same time. The first recognizable stages appear like minute vacuoles frequently situated at the side of the nucleus of the red cell instead of at one end as later. Only one or a few bacilliform bodies may be present at this time (Fig. 6). As growth proceeds the size of the Cytamoeba increases as well as the number of bacilliform bodies. Fully grown parasites differ with respect to the number of these bodies, some being supplied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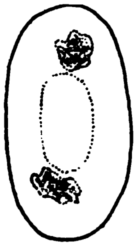
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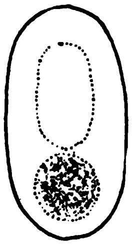
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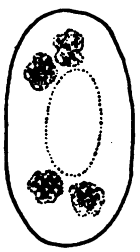
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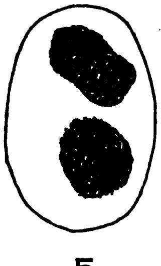
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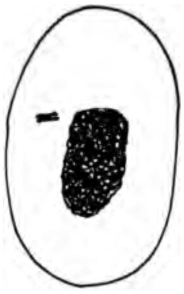
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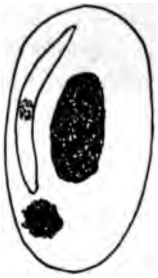
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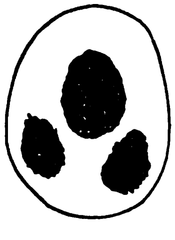
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The name *Cytamoeba bacterifera* we owe to Labbé (1891, 1894). This investigator recognized it as a new type of organism and placed it in the genus Cytamoeba, a generic name proposed previously by Danilewsky (1890) for the malarial parasite. A parasite of the red blood cells of *Hyla viridis* described by Grassi (1882) and considered by him analogous to the malarial organism was placed by Labbé provisionally in the same genus and given the name *Cytamoeba grassi.* Labbé describes what he thought were stages of Cytamoeba in fission and spore formation, and specimens that he found free in the blood.

Wasielewski (1896) accepted Labbé's views regarding the protozoan affinities of *Cytamoeba bacterifera* and placed it in the order Acystosporidia, family Haemamoebidae, along with the genera Proteosoma, Haemamoeba, and Dactylosoma. Cytamoeba was next described by Ziemann (1898) who noted the amoeboid movement of vacuole-like bodies within the red blood cells of the frog, and observed the bacilliform bodies contained in them but was not convinced that the body he saw was really an amoeboid organism.

Laveran (1899) also observed *Cytamoeba bacterifera* both in fresh and in stained preparations. He named the bacilliform bodies *Bacillus krusei* and concluded that the bacteria were not contained in a parasitic ameboid organism, because (1) no movement of the vacuole was observed; (2) the bacilliform bodies moved actively as though in a liquid and not in protoplasm; (3) isolated bacilli were encountered, without a surrounding clear area; (4) the vacuoles did not become stained and no nucleus could be found; (5) after the dissolution of the protoplasm of the red blood cell, the bacteria were isolated; and (6) no reproduction of the "ameba" was observed. Laveran did not succeed in cultivating *Bacillus krusei.* Several of Laveran's contentions do not hold since (1) the "vacuole" certainly undergoes amoeboid movement, (2) protoplasm within certain amoebae is liquid as is probably also true of Cytamoeba and the active movement of the bacilliform bodies is possible in this medium, (3) there is no reason why bacilli should not exist outside of the organism, (4) failure to find a nucleus does not prove that one is not present since it may be obscured by the bacilliform bodies, (5) the protoplasm of the organism as well as that of the red cell may have been dissolved, and (6) the reproduction of the "ameba" may occur in the internal organs.

More recently Dutton, Todd and Tobey (1907) have described several organisms that were found by them in the red blood cells of frogs in Africa, some of which may have been Cytamoebas; and Carini (1910) has attempted to classify similar bodies found in the Brazilian frog, *Leptodactylus ocellatus*. These were apparently observed only in stained specimens and hence were unsatisfactory for study. Certain of them are called by Carini "elements bacilloides"; a second type consists of bacilliform bodies similar to Bacillus krusei but thinner and shorter, and a third type resembles Cytamoeba.

After studying infected red blood cells of the frog both living and fixed and stained and reviewing the literature on this subject the tentative conclusion is reached that *Cytamoeba bacterifera* is a stage in the life cycle of a protozoan parasite and that living within it either as hyperparasites or in symbiosis is a bacillus named by Laveran *Bacillus krusei*. The writer hopes at some future time to add more to our knowledge of this interesting organism.

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AN ANALYSIS OF THE RELATION BETWEEN GROWTH AND NUCLEAR DIVISION IN A PARASITIC INFUSORION, OPALINA SP.[1]

R. W. HEGNER, PH.D.

SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY

AND

HSIANG-FONG WU, M.D.

NATIONAL MEDICAL COLLEGE OF PEKING, CHINA

THIS investigation was undertaken for the purpose of analyzing the relation between growth and nuclear division in a species of *Opalina* of the frog during the growth period in the tadpole. The multinucleate condition of *Opalina* and the absence of cell walls render it of particular value as material for the study of the phenomena involved in nuclear division and growth. The specimens used in our investigations were obtained by Dr. Charles E. Simon from tadpoles collected at Chester, Nova Scotia, during the summer of 1920. Unfortunately we are unable to state either the species name of the *Opalina* or that of the host. Dr. Maynard M. Metcalf, who has examined the slides, thinks the *Opalina* is probably an undescribed species. The material was well fixed in Schaudinn's solution and beautifully stained with iron-hematoxylin.

A sufficiently large number of specimens (455) were drawn with a camera lucida so as to furnish reliable results when measurements were treated by statistical methods. The area of the drawings was determined with a planimeter and the correlation with the nuclear number determined. Table I is the correlation table for the nuclear number and area of 341 specimens. The area of the drawings, which were made at a magnification of 650 diameters, is given in square millimeters. The coefficient of correlation is remarkably high, namely, .755 ± .016; this proves that an increase in size is accompanied by a corresponding increase in nuclear number. The rest of the specimens that were measured, 114 in number, were drawn at a magnification of 1400 diameters. The coefficient of correlation of this lot was found to be .875 ± .015.

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[1] From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

TABLE I

CORRELATION TABLE FOR NUMBER OF NUCLEI AND AREA OF 341 SPECIMENS

The area is given in sq. mm. and obtained from camera-lucida drawings made at a magnification of 650 diameters. Coefficient of correlation .755 ± .016.

Area	Number of Nuclei																	
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	27	28	29	
300+		1																1
400+	1	1	2				1											5
500+		1	1	1	1													4
600+	1	7	7	3	1													19
700+		4	6	12	2													24
800+		2	12	13	5	3		1										36
900+			6	25	8	6	1											46
1000+			1	13	14	9	8											45
1100+		2		3	12	20	6	4	1									48
1200+			1	1	8	3	5	5	1									24
1300+	1	1	1	1	1	5	4	4	1		2							21
1400+				1	1	3	4	5	4	2								20
1500+				1				4	1	1	1	3						11
1600+							1	1	3	2	1		1					9
1700+			1		1		1	1							1			4
1800+			1						2	2					1			6
1900+			1	1		1	1		1	1	1							7
2000+													1					1
2100+									1	1		2						4
2200+									1									1
2400+								1					1	1				3
2800+																	1	1
3200+																1		1
	3	19	40	75	54	50	32	26	16	9	5	5	3	1	1	1	1	341

Metcalf[2] has pointed out that in multinucleate Opalinas the nuclei within a single specimen may be in different stages of division at one time. This we have found to be true also of the nuclei during the growth stages in the tadpole—a condition that has enabled us to analyze with considerable accuracy the exact relation between cytoplasmic mass and nuclear division. For example, among the specimens with four nuclei, there were a few with three "resting" nuclei and one nucleus in division (Fig. 3); obviously one of the four nuclei is undergoing division before its three sisters. If the sum of the areas of a number of specimens in which there are four nuclei of equal size (Fig. 2) is divided by the total number of nuclei, a fairly accurate idea may be obtained of the amount of cytoplasm associated with each nucleus. According to the nucleo-cytoplasmic relation theory[3] an increase in the amount of cytoplasm as compared with the amount of nuclear material furnishes the stimulus which initiates nuclear division. A comparison between specimens with four equal nuclei, and specimens with four nuclei one of which is undergoing division, should reveal approximately the increase of cytoplasmic substance necessary to inaugurate nuclear division. A number of cases of this sort were available in our material and were studied with the following results.

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[2] Metcalf, M. M., 1909, "*Opalina*," *Arch. f. Protist.*, 13: 195–375. Especially p. 269.

Table II shows the relations between area, and number, volume and surface of the nuclei in the 207 specimens that could be used for this purpose. The measurements were made of camera-lucida drawings at a magnification of 650 diameters. Beginning with the group of 15 at the top of the table we can make the following comparisons.

1. Fifteen specimens, each with 4 equal nuclei (Fig. 2), have an average area per nucleus of 176.1 sq. mm.; 10 specimens, each with 3 equal nuclei and a fourth nucleus in division (Fig. 3), have an average area per nucleus of 185.0 sq. mm. The specimens in which division has been initiated have an average area per nucleus 8.9 sq. mm. greater than those with an equal number of nuclei, none of which are in division. We have used the area through-

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[3] For a recent discussion of this theory see Hegner, R. W. 1920, "Relations between Nuclear Number, Chromatin Mass, Cytoplasmic Mass, and Shell Characteristics in Four Species of the Genus *Arcella*," *Jour. Exp. Zool.*, 30: 1–95.

TABLE II

TABLE SHOWING THE RELATION BETWEEN AREA AND NUMBER, VOLUME AND SURFACE OF THE NUCLEI

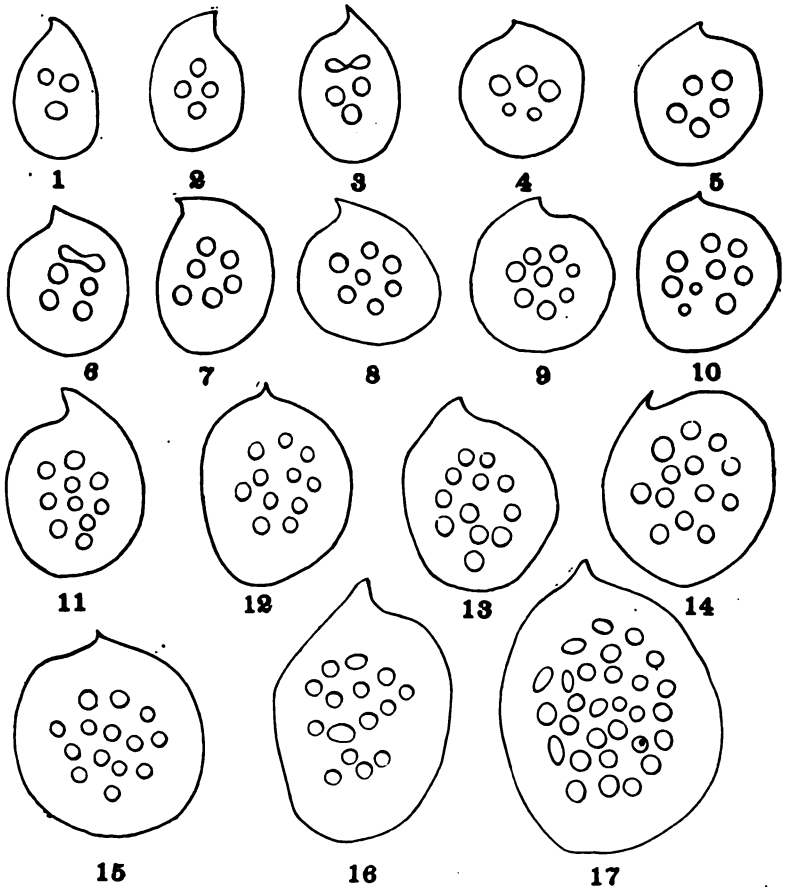
The area, volume and surface of the nuclei were computed from camera drawings made at a magnification of 650 diameters.

Number of Specimens	Number of Nuclei	Average Area in Sq. Mm. at Magnification of 650 Diameters	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters	Average Volume of Nuclei in Cubic Mm. at Magnification of 650 Diameters	Average Volume per Nucleus in Cubic Mm. at Magnification of 650 Diameters	Average Surface of Nuclei in Sq. Mm. at Magnification of 650 Diameters	Average Surface per Nucleus in Sq. Mm. at Magnification of 650 Diameters
15	4 (equal in size)	704.2	176.1	215.49	53.87	261.32	65.33
10...	4 (one in division)	739.9	185.0				
13...	5 (two small)	851.5	170.3	257.11	51.42	321.10	64.22
16...	5 (equal in size)	860.5	172.1	281.91	56.38	345.01	69.00
17...	6 (two small)	951.4	158.6	273.69	45.62	363.85	60.64
49...	6 (equal in size)	926.7	154.5	264.83	44.14	352.89	58.81
5...	6 (one in division)	1,032.5	172.1				
11...	7 (two small)	993.5	141.9				
31...	7 (equal in size)	1,066.7	152.4	269.17	38.45	379.68	54.24
10...	7 (one in division)	1,176.7	168.1				
30...	8 equal	1,127.9	141.0	336.53	42.07	458.93	57.37
207							

out our work as a measure of cytoplasmic mass, hence it appears from the results of our measurements that an increase in mass per nucleus represented by an increase in area within the limits of 8.9 sq. mm. is the stimulus that initiates nuclear division. The exact mass of cytoplasm represented by this increase in area of 8.9 sq. mm. might easily be obtained under more favorable circumstances.

2. When we compare the measurements of the 10 specimens with 4 nuclei, one of which is dividing (Fig. 3) with 13 specimens of the stage immediately following, with 3 large nuclei and two that have just reorganized after division (Fig. 4), we find that although the latter average 111.6 sq. mm. larger per specimen the average area per nucleus is 14.7 sq. mm. less. Thus there has been an

actual increase in size but a decrease in the mass of cytoplasm associated with each nucleus.



FIGS. 1–17. Outline drawings of stages in the growth of *Opalina* sp. made with a camera lucida at a magnification of 650 diameters and reduced to a magnification of 325 diameters.

3. During the growth of the two small nuclei to their full size (Figs. 4–5) the size of the organism increases from an average area of 851.5 sq. mm. per specimen to an average area of 860.5 sq. mm., or an average area per nucleus of from 170.3 sq. mm. to 172.1 sq. mm. Although an increase in size has taken place, the average area per nucleus of 172.1 sq. mm. in specimens containing 5 full-

sized nuclei is less than that of specimens with 4 full-sized nuclei, *i.e.,* 176.1 sq. mm.

4. A further increase in the average size of the specimens occurs between the stage with 5 nuclei of equal size (Fig. 5) and that with 6 nuclei, two of which have just emerged from mitosis. Measurements give for the former an average area of 860.5 sq. mm. and for the latter 951.4 sq. mm. The specimens with the two small nuclei, however, possess as before (see (2)) a lower average area per nucleus, *i.e.,* 158.6 sq. mm. as compared with 172.1 sq. mm. in specimens with 5 full-grown nuclei.

5. The measurements of the next stage, *i.e.,* specimens with 6 nuclei of equal size (Fig. 7), are more difficult to explain, since the average area of the specimens (926.7 sq. mm.) is actually less than that of the younger specimens (see (4)) with 4 large and 2 small nuclei, and the average area per nucleus falls from 158.6 sq. mm. to 154.5 sq. mm. These results may be due to a thickening of the entire animal which would increase the mass and tend toward a decrease in area or the nucleo-cytoplasmic relation may change as the animals become older. That there is an actual decrease in the average area per nucleus as growth proceeds is indicated by the measurements of later stages as given in Table III. This table shows a decrease per nuclear area from 186 sq. mm. in specimens with 4 nuclei to 96.8 sq. mm. in specimens with 29 nuclei. That this decrease is gradual is indicated when averages are made of three successive groups containing each a larger number of specimens. Thus the 4, 5, and 6 nucleated groups containing 134 specimens have an average area per nucleus of 173.8 sq. mm., the 7, 8, and 9 nucleated groups containing 136 specimens have an average area per nucleus of 144.4 sq. mm., the 10, 11, and 12 nucleated groups containing 51 specimens have an average area per nucleus of 143.5 sq. mm., and the 13, 14, and 15 nucleated groups containing 13 specimens have an average area per nucleus of 128.3 sq. mm.

TABLE III

TABLE GIVING THE AVERAGE AREA PER SPECIMEN AND PER NUCLEUS OF 338 SPECIMENS DRAWN WITH A CAMERA LUCIDA AT A MAGNIFICATION OF 650 DIAMETERS

Number of Specimens.	Number of Nuclei.	Average Area per Specimen in Sq. Mm. at Magnification of 650 Diameters.	Average Area per Nucleus in Sq. Mm. at Magnification of 650 Diameters.
19......	4	744	186.0
40......	5	891	178.2
75......	6	944	157.3
54......	7	1,069	152.7
50......	8	1,150	143.7
32......	9	1,231	136.8
26......	10	1,395	139.5
16......	11	1,635	148.6
9......	12	1,709	142.4
5......	13	1,572	120.9
5......	14	1,801	128.6
3......	15	2,032	135.4
1......	16	2,452	153.2
1......	17	1,806	106.2
1......	28	3,277	117.0
1......	29	2,806	96.8

6. By the time one of the nuclei of the six-nucleated stage has been stimulated to division the average area per nucleus increases again to 172.1 sq. mm. After this division is completed and seven nuclei are present, two of them small, the average area per nucleus, as was to be expected, decreased to 141.9 sq. mm. During the period necessary for the two small nuclei to reach their full size (Fig. 8) the area increases again to 152.4 sq. mm. A further increase to 168.1 sq. mm. occurs by the time sufficient growth takes place to stimulate one of these seven nuclei to divide, and a decrease (to 141.0 sq. mm.) again takes place when this stage evolves into that with eight nuclei (Fig. 9).

The two curves in Fig. 18 show clearly the increase in area per nucleus up to the point where one nucleus divides, then a conspicuous decrease following nuclear division, and subsequently an increase during the period when the nuclei resulting from division regain their full size, ending in a size at which the area per nucleus is approximately that present at the beginning.

Figure 19 illustrates the fact that the size of the entire specimens increases during nuclear multiplication and growth, but that the area per nucleus remains almost constant.

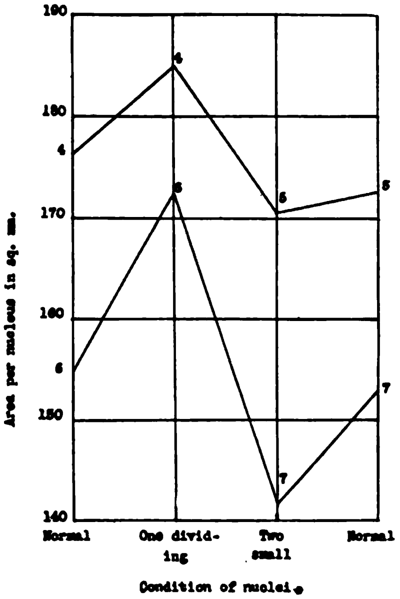


FIG. 18. Curves showing the changes in area per nucleus that accompany changes in nuclear number and condition. The numbers 4, 5, 6, and 7 indicate the number of nuclei present.

7. The series of measurements of these specimens affords an explanation of the reason why nuclear division in *Opalina* is not synchronous. According to the nucleo-cytoplasmic relation theory, as soon as the mass of cytoplasm has increased to a certain point nuclear division is initiated. The necessary increase to furnish this stimulus in *Opalina* may be determined approximately from our data by comparing measurements of specimens in which the nuclei are all equal in size with those in which nuclear division has been inaugurated. Such a comparison gives the following results.

Nuclei	Average Area per Nucleus	Difference in Area — Amount Necessary to Stimulate Division
4 (equal)	176.1 sq. mm.	
4 (one in division)	185.0 sq. mm.	8.9 sq. mm.
6 (equal)	154.5 sq. mm.	
6 (one in division)	172.1 sq. mm.	17.6 sq. mm.
7 (equal)	152.4 sq. mm.	
7 (one in division)	168.1 sq. mm.	15.7 sq. mm.

These figures, of course, indicate only the relative increase necessary to stimulate nuclear division; the actual increase could be determined by measuring accurately the mass of cytoplasm in each case.

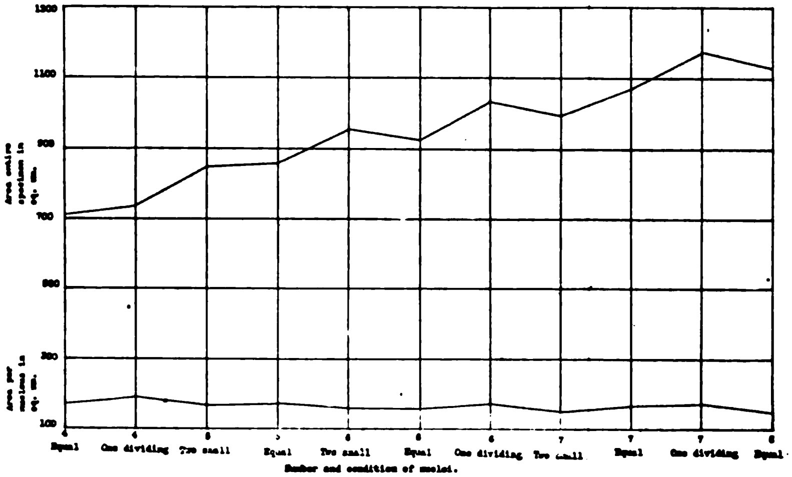


FIG. 19. Curves showing the increase in the area of specimens with increase in age and the constancy of the area per nucleus.

In looking over our camera drawings it was noticed that usually only one nucleus was in division in any one specimen (Figs. 3 and 6) and that in many cases two of the nuclei in a specimen were smaller than the rest (Fig. 4), indicating that they were daughter nuclei that had just emerged from mitosis. Of a total of 137 specimens in which nuclei were found in division, 109 contained one division figure, 19 contained 2, 8 contained 3, and 1 contained 4. Furthermore, those containing more than one division figure were usually older than those containing one only. Thus the average nuclear number of specimens with one dividing nucleus was 7.2, with two dividing nuclei, 8.1, with three dividing nuclei, 9.3, and with 4 dividing nuclei, 10. These data favor the conclusion that the stimulus that initiates nuclear division acts as a rule on only one nucleus at a time and that the division of this nucleus restores the nucleo-cytoplasmic ratio. When this ratio is again disturbed by an increase of the cytoplasmic mass another nucleus is stimulated to divide. Division of two or more nuclei synchronously may be due to the more rapid growth, the larger specimens in which this usually occurs, or to the greater chances of two or more nuclei reacting to the division-stimulus when a large number of nuclei are present in a single specimen. There is some evidence that the nucleus that undergoes division is the one with the greatest amount of cytoplasm surrounding it, but this could not be determined definitely. No regular distribution of the nuclei was evident. It is interesting to note in this connection that during the embryonic development of many animals nuclear division occurs in all cells at nearly the same time. This is especially interesting in the case of certain insects, in the eggs of which nuclear division proceeds synchronously without the intervention of cell walls until thousands of nuclei are present in a single egg.[4] An increase of cytoplasm over nucleus may also, in these insect eggs, stimulate nuclear division, since after each division the mass of cytoplasm surrounding each nucleus is increased by the addition of new material elaborated from the yolk substance in which it is situated.

8. The average total volume of the nuclei of certain specimens, average volume per nucleus, average total area of the surface of the nuclei and average area of the surface per nucleus were measured in cubic millimeters and square millimeters from our camera drawings which

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[4] Hegner, R. W., 1914, "Studies on Germ Cells," *Jour. Morph.*, 25: 375–509. Especially pp. 408–413.

were magnified 650 diameters. These data are shown in Table II. They indicate one point of considerable interest and that is the fact that as the number of nuclei increases their average volume and surface decrease.

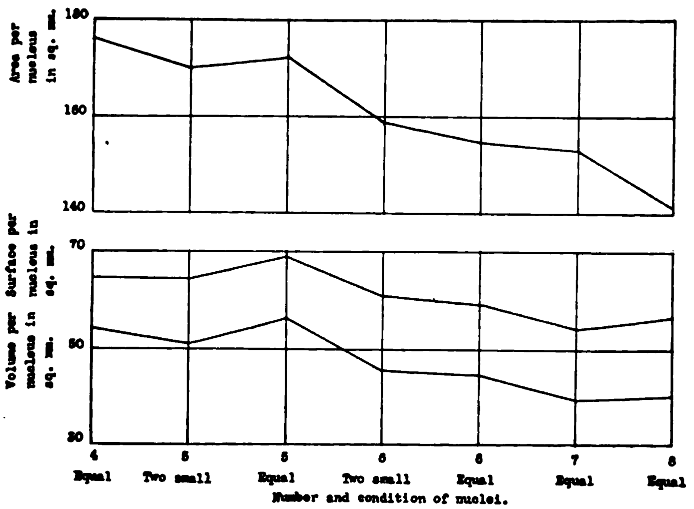

FIG. 20. These three curves show that decreases in the volume and surface of the nuclei are accompanied by decreases in area per nucleus.

Thus in specimens with 4 nuclei of equal size each nucleus has an average volume of 53.87 cu. mm., in specimens with 6 nuclei the average volume per nucleus decreases to 44.14 cu. mm., and in specimens with 8 nuclei the average volume per nucleus decreases still further to 42.07 cu. mm. Similar results were obtained from measurements of the surface of the nuclei, but the decrease is not so great since the volume decreases as the cube whereas the surface decreases only as the square. As the table (II) shows the average surface per nucleus in sq. mm. decreased from 65.33 sq. mm. in specimens with 4 nuclei, to 58.81 sq. mm. in specimens with 6 nuclei, and 57.37 sq. mm. in specimens with 8 nuclei. This decrease in volume and surface may account for the fact noted previously (in (5)), that the area per nucleus decreases in specimens with nuclei all equal in size as the number of nuclei becomes greater with advancing age. Since the volume of the nuclei is less in these older specimens the amount of cytoplasm associated in normal nucleocytoplasmic relations with them is less and the area of the specimens per nucleus decreases accordingly.

The curves in Fig. 20 bring out clearly the relation between area and volume and surface of the nuclei during the growth period. The average area per nucleus decreases as the number of nuclei increases, but at the same time there is a corresponding decrease in both volume and surface of the nuclei, thus maintaining approximately the initial relation between nucleus and cytoplasm.

SUMMARY

(*a*) A high correlation exists between nuclear number and cytoplasmic mass (as indicated by area) during the growth of *Opalina* sp. The coefficient of correlation in one lot of 341 specimens was .755 ± .016 and in another lot of 144 specimens was .874 ± .015.

(*b*) By comparing the area of various stages with the number, size, state of division, volume and surface of the nuclei the following conclusions were reached. (1) Nuclear division is stimulated by an increase of cytoplasm that may be determined approximately. (2) As the organisms increase in age the nuclei decrease in volume and surface; this is accompanied by a corresponding decrease in the area per nucleus, indicating that the nucleocytoplasmic relation is maintained. (3) Nuclear division is not synchronous because one nucleus is usually stimulated to divide before the others, and this division is sufficient for the time to reestablish the normal relation between nuclei and cytoplasm.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.*

I. GENERAL INTRODUCTION.

BY WILLIAM W. CORT, PH.D.

(Received for publication, July 15, 1921.)

A. FACTORS INVOLVED IN HOOKWORM CONTROL.

The gradual accumulation of information on the life cycle of the hookworm has made it possible to apply control measures with increasing precision. Much has already been accomplished, and the experience of men engaged in control work is constantly pointing to problems, the solution of which will make the attack on hookworm disease more effective. It is the purpose of this first article of the series to analyze the factors involved in hookworm control and to suggest certain lines which need further investigation.

To control a disease produced by a parasitic organism it is necessary to find a point at which its life-cycle can be broken. The life-cycle of the hookworm including as it does both a parasitic stage and an active free-living stage offers three points of attack, viz., the parasitic adults, the eggs and the newly hatched larvae, and the infective larvae. As applied to control measures the attempt to kill the parasitic stage of the hookworm includes (1) the recognition, usually by fecal examination, of the individuals who harbor the parasites, (2) the administration of an anthelminthic to infested individuals, (3) the reexamination after treatment to discover if the worms have been dislodged, and (4) the retreatment, until the examination becomes negative, of those individuals who are still infested. The killing of the hookworm eggs can be accomplished immediately by disinfection of the feces containing them, or by the prevention of the deposition of feces by infested individuals under conditions where the eggs can hatch and reach the infective stage. This phase of the attack centers around the prevention of soil pollution, and is also effective in the control of other diseases, such as typhoid fever and the protozoan and bacillary dysenteries. The killing of the infective hookworm larvae may be accomplished directly by disinfecting the soil in which they live, or indirectly by preventing the human host from coming into contact with such soil under conditions which would make infestation possible.

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* The series of papers which will be published under this title represents the results of hookworm investigations carried out by the Department of Medical Zoology of the School of Hygiene and Public Health of Johns Hopkins University with the cooperation of the International Health Board of the Rockefeller Foundation.

It is impossible to outline any standard method of hookworm control which would be applicable under all circumstances since the conditions vary so greatly. It is possible, however, by critical analysis to evaluate in a very general way the measures of control commonly used. First, it must be constantly borne in mind that in a campaign for the control of hookworm disease the object is primarily to kill worms and the results must in the main be judged not so much by the number of patients cured as by the reduction of the total infestation of the region where the work is being done. That the reduction of mass infestation is not necessarily synonymous with patients cured was brought forcibly to my attention several years ago in connection with the hookworm control work in the mines of California. In a mine, slightly out from the endemic center, examinations showed twenty positives out of about one hundred underground workers. All except two of these infestations were very light, showing by the culture method of diagnosis only one or very few larvae from a culture. Almost all of these cases would have been negative if one of the more usual methods of fecal examination had been used. The other two men had extremely heavy infestations which they had evidently acquired in a heavily infested mine in which both had worked three years previously. It was roughly estimated that these two men harbored at least ten times as many worms as all of the other eighteen together. In the report the twenty cases were marked positive and looked much alike, but for the control of the disease in the mine the two heavily infested individuals were much more important than all of the other eighteen put together. Especially was this true since one of them had habitually defecated in a sump which was visited by the other workers and which was literally swarming with hookworm larvae.

A second general consideration in connection with hookworm work is that each control project differs from every other and therefore must be attacked as an independent problem. The factors which must be considered in determining the best method of procedure in any given case are so complex that the director of the campaign must not only investigate the conditions carefully before planning his work, but must be constantly alert to vary his methods as new information makes it seem desirable. The best methods to be used in any given hookworm campaign depend among other things on the purpose of the work, the resources available, the character and customs of the people, the conditions under which they live, the degree of infestation and the chief sources of infestation. For example, if a plantation owner with a shifting labor population wishes to increase the efficiency of his labor, he will be interested chiefly in the reduction of mass infestation in his workers, and will pay special attention to the sources of infestation on his plantation. A medical man working alone with a small appropriation would hardly concern himself with the improvement of his method of examination or with completely curing his patients, but would try to treat the worst cases and spread as much information as possible. Where human excrement is used for fertilizing the soil the methods of attack become quite different from a region in which this practice does not exist. The hookworm campaigns carried on under army conditions assumed an entirely different aspect from those in a civil population, because it was possible to absolutely control the individuals with whom the work was being done. Examples of this kind could be multiplied, but those given serve to illustrate the futility of trying to work out by rule of thumb any best program of hookworm control.

In the inauguration of a hookworm campaign the discovery of the exact sources of infestation is of great importance. Without this information the control measures in a region can only be directed at a general reduction of mass infestation. The importance of developing methods for making definite epidemiological studies on hookworm disease is emphasized by the helplessness many workers feel when they find a high degree of reinfestation of the population of a region only a short time after the conclusion of an intensive hookworm campaign. That it is feasible in connection with a hookworm campaign to study intensively the sources of infestation is well illustrated by the work of Baermann (1917)[1] in Sumatra. This investigator has worked out a simple method by which infective hookworm larvae can be isolated from soil (Baermann, 1917).[2] By the use of this method he has apparently been able to uncover the chief sources of infestation in the area in which he was working. He was led to make these studies by the fact that even after applying the ordinary measures for a period of several years there was a considerable degree of infestation which he was unable to eradicate. I will not go into the many interesting details of his work, but will only mention his finding of one entirely unsuspected source of infestation. He found hookworm larvae in large numbers in damp areas on the dirt floors of the houses around the places where the washing and drinking water was kept. He suggests a number of ways by which the larvae might have been brought to these places, and concludes that these spots were the chief source of infestation for the people living in the houses. Here then was an important source of infestation the detection of which at the beginning of the work would have made the whole campaign more effective.

In analyzing the factors involved in hookworm control the phases of the work connected with the killing of the parasitic stage, or in other words, the treatment, will be first discussed. Before treating, examinations are usually made to find the individuals carrying the infestation. Certain workers have advocated the omission of the diagnostic examinations and the treatment of the whole population where a preliminary survey has shown a very high mass infestation. Such a procedure might be justifiable where over eighty per cent. of the population are infested and would result in a considerable saving. The factors involved in the choice of any particular method of fecal examination for the diagnosis of hookworm disease are so complex that it is not possible to say that any given method is the best for all circumstances. The choice of method must depend on the purpose of the work, the number of examinations to be made, the money available, the conditions under which the work is to be done, the qualifications of the microscopists, etc. It is practically impossible to make the diagnostic examination so critical that every individual who harbors hookworms will be marked positive. Also, in a certain number of the cases the number of worms present is so small that such individuals could hardly be considered an appreciable factor in the spreading of the disease. The method of examination used should certainly be sufficiently accurate to find all those who harbor a sufficient number of worms to produce clinical symptoms, as well as those who have a considerable degree of infestation but high resistance. In most campaigns the accuracy of the examination can be carried far beyond this point, but it should not be carried to the extreme where the increased cost in time and money is out of proportion to the results obtained.

In the treatment phase of the work quite a proportion of the time and money expended often goes to the attempt to "cure" the patients treated. Sometimes four or five treatments and the corresponding reexaminations are given a single patient before he is pronounced "cured," the term "cured" being applied when the number of worms has been reduced below the point where the method of examination in use gives a positive finding. It is evident that where less critical methods of reexamination after treatment are used there will be a larger number of "cures." On account of this possible source of error and the time consumed there has been a tendency recently to question the value of this phase of the work. Perhaps further experiments will make it possible to standardize so definitely the methods of treatment for hookworm disease that all reexaminations and subsequent retreatments will become unnecessary. Certainly the expenditure of considerable portions of moneys available for hookworm control work in attempts to clear out the last few worms from the patients is not justifiable when we consider the millions suffering from hookworm disease who cannot be reached by any work now organized.

In the attempt to break the life-cycle of the hookworm by killing the eggs, control work has been centered around the prevention of soil pollution. This phase usually consists in seeing that the people are provided with proper latrines and are educated to use them. In many ways this is the easiest and most effective measure for hookworm control and without its inclusion no campaign can have lasting value. It has the advantage of being also an attack on the numerous other diseases which are spread by soil pollution. In this phase of the work there are two critical points. First, it must be certain that the type of fecal disposal which is advocated really prevents the spread of the disease. Second, the whole success of this phase of the work rests on whether the people of the area will be sufficiently enlightened to cause an appreciable number of them to continue to maintain and use the latrines after the conclusion of the campaign. Unfortunately in many areas where hookworm work has been carried on there has not been any great decrease in soil pollution, and time after time even where proper latrines are installed in adequate numbers only a short time elapses before the people have returned to their former habits of soil pollution. In regions where the population is very primitive or where human excrement is used for fertilizing the soil the problem seems almost incapable of solution. The habits of people are not easily changed. Only by educational activities carried out by governmental agencies over a term of many years and supplemented by carefully planned and adequately enforced legislation can it be hoped to permanently improve in many places the methods of excreta disposal. To prevent pessimism in regard to present hookworm control campaigns it should be remembered that in many cases they are merely entering wedges designed to start in motion those larger agencies that may in time be able to raise the standards of the people.

Connected with the prevention of soil pollution in the general educational campaign is the question of the prevention of the entrance of the infective larvae into man. The manifold phases of this need not be discussed here, but it is necessary to throw out the caution that since in most areas the actual sources of human infestation are not known, it is only when careful epidemiological investigations are carried out that information of more than a very general nature can be given to the people for their personal protection.

There has been considerable discussion and much experimentation on possible methods of killing hookworm eggs and larvae in the soil. As compared with bacteria, the infective hookworm larvae are surprisingly resistant to disinfectants. In considering the effect of various chemical reagents on hookworms in the soil it must be remembered that all stages of the hookworm in the soil are not affected in the same way. Experiments have shown that any given chemical agent which might be used for disinfection acts differently on the eggs, the newly hatched larvae and the infective larvae. The newly hatched larvae are much more easily killed than either of the other stages. It is apparently not practicable to disinfect generalized sources of infestation. It is possible, however, that this method of hookworm control has a much more important application than has been realized. Certainly it is possible to disinfect limited areas such as highly polluted mine "sumps," the areas around faulty latrines, or infested places in a house or yard. Unfortunately our knowledge is still unsatisfactory in regard to the best disinfectants to use and their method of application.

B. PROBLEMS IN HOOKWORM CONTROL.

There is available a large body of information both on the life-history of the hookworm and the methods of hookworm control. There is, however, no stage of the life-cycle of the hookworm or no phase of hookworm control work on which further investigations cannot be carried out with profit either from the biological or from the practical point of view. The problems which need investigation in connection with the improvement of hookworm control work can for the sake of convenience be divided into two general categories. The first involves researches on methods of control and the second researches on the biology of the hookworm.

Researches of the first type have for their purpose the reduction in the cost of the methods used or the increase of their effectiveness. When the large sums of money which are being spent in hookworm control and the vastness of the field yet untouched are considered it can be realized how important is any saving or improvement in method, however slight. Researches on improvement of methods can usually be best carried on by men connected with active control work in the field, and from such men results are constantly coming which have added much to the effectiveness of hookworm control work. The director of a hookworm campaign who adequately grasps the significance of his project as an individual research problem can hardly fail to add materially to the improvement of the methods of work. The differences of opinion which have arisen show that there is still plenty of room for further investigations of this type. For example, much can be expected from further comparative studies of the methods of fecal examination and their adaptation to varying conditions. In spite of all that has been done, the treatment of hookworm disease still needs investigation, and progress can be expected both from the comparison and improvement of methods now in use and from the search for new ones. The further development of methods for the disposal of human excreta which can be applied to various regions where soil pollution is prevalent is a promising field. Along this line the U. S. Public Health Service is devoting large sums of money and the services of a number of experts in an attempt to devise methods for the disposal of human excreta which will be adapted to conditions in the Southern United States. Little that is satisfactory is yet known about the disinfection of the soil. In fact every step in hookworm control work still needs to be carefully investigated from a variety of different viewpoints and under different conditions.

In spite of the great mass of published data which is available on the life cycle of the hookworm, it still offers many unsolved problems. While any new information on any phase of the biology of the hookworm may prove of practical value in the attack on th organism, it is that phase of the life-cycle which is passed outsid of the human body which offers the most promising field. Since th investigations which we are undertaking center around the life o the hookworm eggs and larvae in the soil a more extended analysis of the unsolved problems of this stage and their practical applications will be made.

From the biological point of view the investigation of the life of the hookworm in the soil is primarily an ecological problem involving as it does a complete study of the influence of all the various factors of the environment on this phase of the life of the organism. From the point of view of control this problem centers around the question of the infectivity of the soil. It involves a study of the distribution of the hookworm eggs and larvae outside the human body, especially their distribution to situations where they will have a chance to reenter the human body, and of measures which could be employed to keep them from such situations. Such investigations include those which have to do with the attempt to increase knowledge concerning the general relation to environment and activities of this phase of the life-cycle of the hookworm and those which are centered around the problems of the source of human infestation in particular areas. There is really very little precise information on the life of the hookworm in the soil, and seldom has the infectivity of the soil in any given area been scientifically studied. The importance of such studies is apparent when we consider, as was shown earlier, that unless the exact sources of infestation of the people in a given area are known, only general control measures can be applied.

In order to more clearly define the unsolved problems in connection with the life of the hookworm in the soil I will present in outline the phases which need investigation and discuss the practical applications of some of the problems suggested.

Certain phases of an investigation of hookworm in the soil.

I. The eggs and young larvae.
    A. The distribution of the eggs over the soil.
        1. By man.
            a. By direct soil pollution.
            b. By accidental carriage on feet and hands.
            c. By use of human excreta in fertilizing the soil.

2. By animals other than man.
   a. By feeding on human feces.
   b. By human feces clinging to parts of the body.
3. By physical agencies.
   a. By rain.
   b. By bodies of water.

B. Conditions necessary for the development of the hookworm in the soil.
1. Length of time under varying conditions that the hookworm eggs will remain alive without hatching in human stools.
   a. In stools on the soil.
   b. In stools in water.
   c. In stools in various types of latrines.
   d. In buried feces.
2. Hatching of eggs and development of larvae under various environmental conditions.
   a. Will hookworm eggs hatch in buried feces?
   b. Hatching and development in various types of latrines.
   c. Conditions necessary for hatching and development when deposited on the soil or in water.
      (1) Food.
      (2) Moisture.
      (3) Temperature.
      (4) Chemical conditions.

I. The infective larvae.

A. Length of life.
1. Under conditions favorable for great activity.
2. Under conditions which favor inactivity.
3. In various types of soils.
4. Length of time various soils remain infective after pollution stops.

B. Conditions necessary for life.
1. Amount of moisture needed.
   a. Effect of large amounts of moisture.
   b. Resistance to desiccation.
2. Chemical relations.
   a. Favorable and unfavorable chemical situations in natural environment.

        - *b*. Effect of various chemical agents.
        - *c*. Disinfection of infested soil.
- *C*. The spread of the infective larvae.
    - 1. Active migrations.
        - *a*. Horizontal migrations from pollution centers.
            - (1) Effect of temperature.
            - (2) Effect of moisture conditions.
            - (3) Effect of physical character of the soil.
            - (4) Effect of chemical character of the soil.
            - (5) Effect of vegetation.
        - *b*. Vertical migrations.
            - (1) Depth of vertical migrations under favorable conditions.
            - (2) Possible upward migration when larvae are buried.
            - (3) Possible downward migration when upper surface of soil dries.
    - 2. Passive spread of infective larvae.
        - *a*. By man.
        - *b*. By animals other than man.
        - *c*. By physical agencies.
            - (1) By rain.
            - (2) By streams of water.
    - 3. The distribution of infective larvae in an infested area.
        - *a*. With respect to centers of soil pollution.
        - *b*. In relation to disposal of human wastes.
        - *c*. With regard to sources of human infestation.

I shall not attempt to discuss all of the problems which are suggested by this outline, but will merely indicate the importance of the solution of some of them. In the epidemiological study of any given area, the study of the centers of soil pollution and the analysis of the factors involved in the spread of the feces containing eggs would naturally be the first step. In certain regions chickens, dogs, and pigs ingest human feces in considerable quantity. While the rôle of these animals in the spread of hookworm disease has been suggested frequently, their real significance has not been sufficiently investigated. A more accurate understanding of whether these and other animals can spread ingested hookworm eggs or larvae would make it possible to determine whether their activities in any given region were producing definite centers from which the people were being infested.

After an analysis of the spread of the feces containing eggs would come the study to determine just where the eggs find conditions favorable for their development and hatching. Unfortunately, our information is not very clear on a number of factors involved in this phase. Several insufficiently answered questions center around the question of how long hookworm eggs live when they are deposited under conditions unfavorable for their development. The fact has been established that hookworm eggs can remain viable for considerable periods when conditions are unfavorable and then when conditions become favorable can continue their development. It is probable that this power of resistance is partly responsible for the much greater incidence of hookworm than of Strongyloides stercoralis where these two organisms live side by side. To evaluate this factor in any given situation more information is needed to answer such questions as the following: How long can hookworm eggs resist desiccation in a stool deposited on the surface of the soil? How long can eggs remain capable of hatching under conditions such as those within a solid stool or a buried stool in which the supply of oxygen is not favorable for development? A very practical question in this connection is whether the eggs remain viable for any considerable time and especially whether they can hatch and the larvae develop under the conditions found in various types of latrines. Also, whether larvae which have developed in latrines can escape to situations where they would be dangerous to man. Many more investigations are needed on the factors involved in the hatching of the hookworm eggs and the development of the larvae to determine the conditions necessary for such development under natural conditions.

The literature contains a number of references to observations on the length of life of hookworm larvae in the infective stage. In this connection the particular point which needs further investigation is how long after the removal of the sources of soil pollution can a given area remain infective. That is, how long do larvae live under various situations in such numbers as to be an appreciable source of infestation to the population. Since the infective hookworm larvae depend for their food and oxygen on stored material, it seems very probable that the more active they are, the sooner they will die.

We know very little of the wide variety of factors which influence the life of the hookworm larvae in the soil, and further studies are needed on such questions as resistance to chemicals, soil preference, etc. The evidence indicates that desiccation quickly kills the hookworm larvae. An important series of investigations which are greatly needed are those which would determine the minimum of moisture which must remain in the soil of various types to keep the hookworm larvae alive. Further, can hookworm larvae migrate down into the lower layers of the soil as the upper layers become dry? Both of these problems touch the practical question of whether hookworm larvae can live over the dry season in certain tropical regions.

There are few other questions on the biology of the hookworm on which we have less accurate data than on the migrations of the infective larvae. It is not so much, how far theoretically they might migrate, as how far they actually do spread under various conditions from the centers of soil pollution. Studies under both laboratory and field conditions are needed along this line, involving such questions as the influence of the physical and chemical character of the soil and its moisture content on such migrations.

Most of the problems which have just been discussed are related to the question of the sources of human infestation. Such problems are not easily solved, and many of them depend on the development of methods which make it possible to discover whether encapsuled hookworm larvae are present in the soil. On this point Baermann (1917)[2] has been most successful by utilizing the fact that the larvae will leave soil for water. In the work which we are undertaking on some of these problems a modification of Baermann's apparatus has been used for isolating infective hookworm larvae from the soil, and a description of it will appear in number two of this series. Such an analysis might be carried much further, but it has been sufficient to define the problems of the present series of investigations, and possibly to suggest to other workers lines of research which might be followed with profit.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

II. THE DESCRIPTION OF AN APPARATUS FOR ISOLATING INFECTIVE HOOKWORM LARVAE FROM SOIL.*

BY WILLIAM W. CORT, PH.D., JAMES E. ACKERT, PH.D., DONALD L. AUGUSTINE, B.S., AND FLORENCE KING PAYNE, M.D.

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INTRODUCTION.

Little information is available on the life of mature hookworm larvae in soil on account of the difficulty experienced in studying them in their natural environment. Not until the investigations of Baermann (1917) was an apparatus devised with which hookworm larvae can be isolated from considerable quantities of soil. This apparatus makes it possible to study the activities of the infective hookworm larvae in the soil and to determine accurately the sources of infestation with hookworm disease in any region. A modification of Baermann's apparatus has been used with success in the investigations of the past summer. On account of the importance of this apparatus and because Baermann's description is published in a rather inaccessible place we will describe in detail the apparatus which we used for the isolation of hookworm larvae from soil and the experiments which were performed to standardize it under different conditions of soil, moisture and temperature.

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* This paper is the first report from the investigation in Trinidad, British West Indies, on the life of hookworm eggs and larvae in soil, carried on by the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University, with the cooperation of the International Health Board of the Rockefeller Foundation. The expedition worked in connection with the intensive hookworm campaign in Trinidad of which Dr. George C. Payne of the International Health Board was director, and was under the direction of Dr. William W. Cort of the Johns Hopkins University. The expenses of the expedition were paid by the International Health Board. The location of the work in Trinidad was made possible by the interest and cooperation of the Government of Trinidad and Tobago, who through the Surgeon-General, Dr. K. S. Wise, and other officers tendered to the expedition many courtesies which aided in the successful conduct of the work. Those who took part in the investigations were Dr. James E. Ackert, of the Kansas State Agricultural College; Mr. D. L. Augustine, of the Johns Hopkins University; Dr. Florence King Payne, Dr. George C. Payne and Dr. William W. Cort.

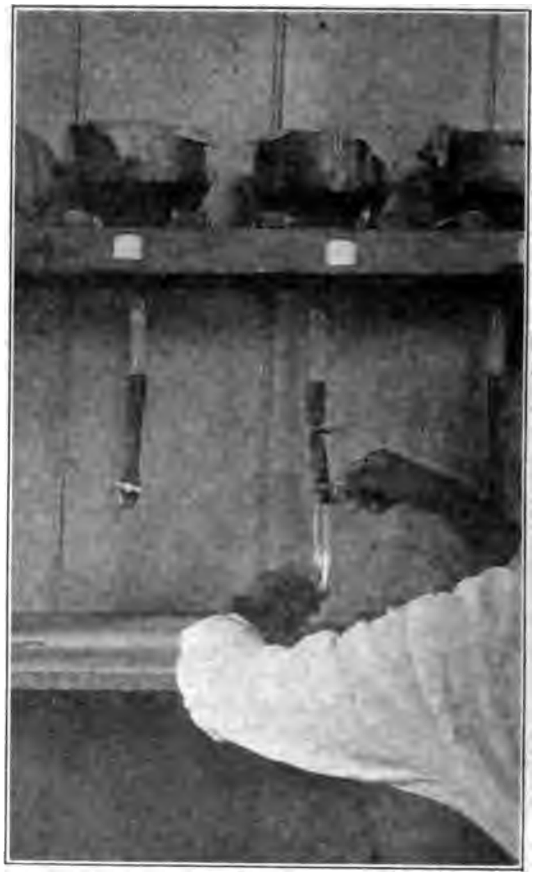

FIG. 1. Two units of the isolating apparatus, showing method of drawing off the water sample.

DESCRIPTION OF ISOLATING APPARATUS.

The apparatus for the isolation of hookworm larvae from the soil works by bringing the lower surface of a soil sample into contact with water of a considerably higher temperature. Under these conditions a large proportion of the nematodes in the sample will pass into the water and can be collected and counted. This isolating apparatus consists of a glass funnel almost filled with water, which has the outlet closed by a clamped piece of rubber tubing. The soil sample is placed in a sieve, which is then fitted down into the funnel so that the level of the water is above the lower surface of the soil. For the examination of soil samples of half a pint or more there were used large glass funnels eight inches in diameter and specially prepared brass sieves seven inches in diameter and three inches in height, with a 1 mm. mesh. To prevent small particles of soil from sifting through into the funnels the sieves were lined with one or two thicknesses of cloth. Figures 1 and 2 show the isolating apparatus set up for use. It will be noted that tightly fitting rubber tubes closed with Hoffman clamps are placed on the stems of the funnels. Nineteen units of this apparatus were in constant use during our investigations. For convenience, as shown in the figures, supports of suitable height were made, and the funnels held in place by padded cross bars. It was found early in the work that if the cross bars were so far apart that the funnels were supported by their rims, considerable breakage re-

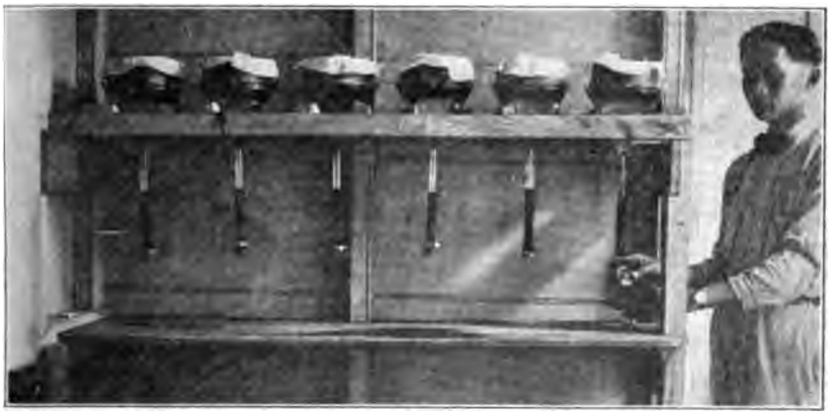

FIG. 2. Six units of the isolating apparatus, showing method of support.

sulted. It is possible to substitute for the sieve a piece of wire screen covered with cloth, of such a size that it can be fitted down into the funnel. The sieves have the advantage of being more easily handled in the transfer of soil.

TECHNIC OF USING THE ISOLATING APPARATUS.

In our routine procedure the soil was thoroughly broken up before being emptied into the sieves. Precautions should be taken not to carry over a little of the soil from one sample to another, and to keep the bottom of the sieve from contact with any dirt. The water used in the funnels was usually heated to a temperature of about 115° F. A funnel, after being set up, was filled within an inch and a half of the rim, so that when the sieve was placed in position there would be no contact between the water and the bottom of the sieve. Water of the same temperature was then poured over the rim of the funnel, until it was practically full and the level of the water almost an inch above the bottom of the sieve. The samples were usually set up in the isolating apparatus some time during the day and left standing over night before the examinations were made. One method of drawing off the samples of water for microscopical examination was to attach a second clamp higher on the tube and to allow the water between the two clamps to run off into a 50 c.c. centrifuge tube (see Fig. 1). A simpler method is to slightly open the clamp at the bottom of the rubber tube and let the water run out until the centrifuge tube is filled. After centrifugation the supernatant water was drawn off with a long pipette and the residue spread out on a two-by-three-inch slide, for microscopical examination. In some instances, when the soil sample was of saturated clay or very finely divided sand, a considerable amount of sediment would fall into the funnel. This could, if necessary, be reduced in amount by running it through a smaller isolating appartus before microscopical examination.

For the examination of small soil samples it was found to be convenient to use a funnel about 9 cm. in diameter. Since no small sieves were available, pieces of screen, bent into a cone shape and lined with cloth, were used for holding the soil samples. The smaller apparatus effected a considerable saving of time in the examinations of small samples, and the standardization tests with it (see Table IV) were somewhat better than those with the large apparatus.

IDENTIFICATION OF MATURE HOOKWORM LARVAE.

In samples taken in the field, or from experiments where the soil had not been previously heated, there were almost always present nematodes other than hookworm larvae. Baermann (1917 *a*, p. 629) used about one to five drops of a 7.5 per cent. solution of caustic soda to kill these nematodes in the microscopical preparations. We found that a few drops of 5 per cent. formalin, mixed with the water on the slide, would kill the unsheathed nematodes, while the mature hookworm larvae, both sheathed and unsheathed, would remain active for some time. It was not enough, however, after such treatment merely to count the actively moving worms, since the unsheathed hookworm larvae soon quieted down, and sheathed larvae of other kinds were often present which remained active. Since mature unsheathed hookworm larvae were found in large numbers in soil, both under natural and experimental conditions (see Cort, et al., 1922), it was necessary to learn to identify accurately both the sheathed and unsheathed forms. The mature hookworm larvae, both before and after losing their sheath, can be definitely distinguished from all the other kinds of nematodes which have been present in the soil samples.* They show considerable variation in size, but their structure and movements are characteristic. The points of structure which were found most helpful in identifying mature hookworm larvae were (1) the shape and relative length of the esophagus, (2) the general shape of the body, (3) the position of the anus and shape of the tail, (4) the characteristic buccal cavity, (5) the small size of the reproductive primordium, and (6) the peculiar refractivity of the cells of the intestinal wall which gives these worms a color and sheen different from any of the other nematodes found. Besides, the movements of both the sheathed and unsheathed hookworm larvae are very characteristic. We found it helpful at first to keep constantly at hand preparations of hookworm larvae from cultures, to compare with doubtful forms from the soil samples. Later, however, we became so well acquainted with hookworm larvae that there was no further difficulty in making the distinction. The difficulty which certain observers seem to have encountered in distinguishing hookworm larvae from other nematodes can easily be obviated by a sufficient study of their structure and movements, before taking up work requiring indentification.

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* Since *Ancylostoma duodenale* is very rare in Trinidad we can assume that the great majority of the hookworm larvae found belonged to *Necator americanus*.

METHODS OF CULTURING HUMAN FECES TO SECURE HOOKWORM LARVAE.

By the technic described above almost a thousand soil samples, both from the field and from experiments, were examined for mature hookworm larvae during the investigations of our expedition in Trinidad. In order to determine the accuracy of the isolating apparatus with different types of soil and to discover the conditions under which it worked best, a number of experiments were performed on soil samples containing known numbers of mature hookworm larvae. For these and other experiments it was necessary to have available large numbers of mature hookworm larvae. The method by which such large numbers of these larvae were obtained in condition suitable for use may be of help to others undertaking similar investigations so that they will be described briefly at this place.

It was possible, in the locality where the work was carried on, to obtain feces from individuals heavily infested with hookworms in sufficient quantity for all purposes, therefore the chief problem which presented itself was the finding of a satisfactory method of culture. At first the cultures were all made with animal charcoal (bone-black), according to Looss's method, in Petri dishes or large soup plates. This method proved unsatisfactory. The culture mass was invaded by fly larvae, especially those of small fruit flies, and in many cases was spread all around in the dishes. In some of the Petri dishes the wanderings of the fly larvae sealed the dishes and kept out sufficient air for the development of the hookworm larvae. Also, the breaking up of the cultures by the fly larvae, both in the Petri dishes and soup plates, often made it impossible to wash the hookworm larvae out into water, without mixing in quantities of bone-black. Further, the making up of a sufficient number of bone-black cultures to obtain the large numbers of larvae needed in the work proved to be a disagreeable, tedious and time-consuming process. After the work had progressed sufficiently to show the difficulties of the bone-black cultures, one of us (Ackert) devised a much more satisfactory method of obtaining the larvae needed. This method can only be used in connection with some such isolating apparatus as that just described. It is much less time consuming than the bone-black culture, and yielded a much surer supply of hookworm larvae. As a culture medium, soil from the yard back of the laboratory was used. This soil was first heated to a temperature of 150° F., to insure the killing of all nematodes, and was placed to the depth of about two inches in shallow tin pans.

eleven inches in diameter, with holes punched in their bottoms to permit drainage. To make the culture the stool was spread out in a layer about one fourth of an inch in thickness on the surface of the soil in the pan and covered with about 1 mm. of wood or charcoal ashes. The sprinkling of ashes over the stool inhibits the growth of mould and also prevents the formation of a hard crust. These cultures should be kept in a screened place, protected from rats and other animals, and must be kept well moistened. Under the conditions in Trinidad the hookworm larvae reach maturity in five days. To isolate the mature larvae the top one half inch of the culture is scraped off and placed in the isolating apparatus. Under tropical conditions only. feces which have not become contaminated with free-living nematodes are satisfactory for culture purposes, because, if the free-living nematodes get started in the cultures, they develop with such rapidity that the hookworm larvae either fail to develop in large numbers or are difficult to use. The soil-ashes method of culturing, if rightly handled, will yield an abundance of hookworm larvae. In one month we made 26 of these cultures which yielded a total of over 36,500 mature hookworm larvae. Four cultures yielded more than 3,000 larvae each. Six of the twenty-six cultures were unsatisfactory, producing less than 200 larvae. This method was certainly employed much more advantageously than the animal-charcoal culture method, since it took much less time to prepare the material and yielded a much higher percentage of successful cultures.

STANDARDIZATION EXPERIMENTS ON THE ISOLATING APPARATUS.

Baermann (1917 a) performed a series of thirteen experiments to test the accuracy of the isolating apparatus which he devised. In his early experiments he used a sieve 7.5 cm. in diamter and 3.5 cm. in height, placed in a small funnel, and studied the results obtained in attempting to isolate larvae from small amounts of soil (50 cc. to 100 cc.). He later used a much larger sieve and funnel for the examination of the samples taken from the field with, as he states, uniformly satisfactory results (Baermann, 1917 b). The results of his experiments with the small apparatus were very satisfactory. In one case he was able to recover seven out of eight larvae placed in the soil. Baermann further found that the mature hookworm larvae travel through the loose soil in the sieves at the rate of 1 cm. in two minutes, and that they fall through the water in the funnel at the rate of

1 cm. per second. In one of his experiments the larvae began to appear at the bottom of the rubber tube in three minutes after the experiment was set up, and the majority were recovered in half an hour. He also found that it was difficult to isolate the larvae from compact coherent soil unless sufficient water was present to immerse the soil and cause it to crumble.

We found it necessary to carry the standardization of the isolating apparatus much farther than Baermann did, and are able to add a number of points to the important findings which he recorded. At the beginning of the work in Trinidad two of us (Cort and Augustine) began a series of experiments to determine the accuracy of the apparatus. Although the apparatus we used conformed as closely as possible to that described by Baermann, and although we had obtained some fairly satisfactory results in Baltimore, the early experiments gave such variable results that they were uniformly unsatisfactory. Finally one of us (Payne) attempted to stimulate the larvae in the soil samples to greater activity by the use of warm water. The higher percentage of larvae which she obtained led to experimentation along this line with satisfactory results. We have since found that when the temperature of the water used in the funnels is lower or only slightly above that of the soil the percentage of larvae isolated is very low (see Table I). This explains the unsatisfactory results of our first experiment, since the water was often used directly from a cistern and was about 2° F. below the temperature of the soil. Baermann makes no mention of the temperature of the water used in his experiments and subsequent soil examinations. Our results indicate that he must have been using water appreciably warmer than the soil to obtain the satisfactory results which he reported.

We will record here the results of the experiments performed, after the apparatus was working satisfactorily, to test the influence of the temperature of the water, the type of soil and the degree of moisture of the soil on the accuracy of the results. We also performed experiments to test the amount of time needed for the larvae to escape from the soil into the water.

Table I gives the results of a series of experiments performed by two of us (Ackert and Cort) to determine the accuracy of the large isolating apparatus with clay loam soil, with different degrees of moisture and with the water in the funnels at different temperatures. The soil used was taken near our laboratory and was the common type found in cultivated areas in the part of Trinidad where we were working. The technic of handling the apparatus in these experiments was the same as that described earlier in this paper. The amount of dirt used was one pint, which was broken up uniformly and spread out evenly in the sieves. The larvae were counted on slides and washed onto the upper surface of the soil in the sieves. This necessitated their migration through the whole depth of the soil in the sieves, making the conditions more difficult than in the samples taken from the field. The soil was either moist or saturated. The temperature of the water was taken just before the sieves were placed in the funnels. Several significant points are brought out by Table I. In the first place it shows that under favorable conditions with the large isolating apparatus at least 50 per cent. of the larvae present will be regularly recovered. Further, it is clearly shown that to obtain satisfactory results the temperature of the water must be at least 10° F. higher than that of the soil (Table I, *c* and *d*). In the next place an examination

TABLE I.

EFFECT OF TEMPERATURE AND MOISTURE ON PERCENTAGE OF RECOVERIES IN CLAY LOAM.

(a) Temperature of soil 82°–84° F. Temperature of water 120° F.

Moist.				Saturated.			
Test No.	No. larvae put in.	No. larvae recovered.	Percentage recovered.	Test No.	No. larvae put in.	No. larvae recovered.	Percentage recovered.
1....	104	69	66.2	16...	120	35	31.8
2....	122	45	36.8	17...	113	47	41.5
3....	106	41	38.6	18...	185	64	34.6
4....	103	40	38.8	19...	90	50	55.5
5....	112	36	32.1	20...	153	23	15
6....	100	32	32	21...	108	28	25.9
7....	100	45	45	22...	99	40	40.4
8....	117	26	22.2	23...	177	98	55.4
9....	106	70	66	24...	155	68	43.2
10....	104	103	99	25...	168	51	30.3
11....	146	113	77.3	26...	142	30	21.1
12....	118	105	88.5	27...	110	68	61.8
13....	145	73	50.3	28...	176	19	10.7
14....	184	139	59.2	29...	93	7	7.5
15....	130	68	40				
			Av. percentage, 52.8				Av. percentage, 33.9

(b) Temperature of soil 80° F. Temperature of water 90° F.

30....	105	57	54.2	39...	110	46	41.8
31....	111	54	48.6	40...	130	65	50
32....	98	37	37.7	41...	122	64	52.4
33....	94	73	77.6	42...	94	38	40.4
34....	138	58	42	43...	163	93	57
35....	140	64	45.7	44...	135	51	37.7
36....	107	56	52.3	45...	140	76	54.3
37....	148	105	70.9				
38....	124	55	44.3				
			Av. percentage, 52.5				Av. percentage, 47.3

(c) Temperature of soil 84° F. Temperature of water 89° F.

Moist.				Saturated.			
Test No.	No. larvae put in.	No. larvae recovered	Percentage recovered.	Test No.	No. larvae put in.	No. larvae recovered.	Percentage recovered.
46....	105	33	31.4				
47....	108	42	38.8				
48....	90	17	18.8				
49....	98	24	24.4				
50....	111	14	12.6				
51....	90	18	20				
52....	105	17	16.1				
53....	103	17	16.5				
54....	93	6	6.4				
			Av. percentage, 20.5				

(d) Temperature of soil 81° F. Temperature of water 79° F.

55....	115	15	13	64...	129	5	3.8
56....	163	15	9	65...	129	11	8.5
57....	205	38	18.5	66...	261	33	12.6
58....	145	14	9.6	67...	176	10	5.6
59....	131	7	5.4	68...	144	11	7.6
60....	130	10	7.6	69...	256	6	2.3
61....	243	25	10.2	70...	146	16	10.9
62....	95	12	12.7	71...	126	12	9.5
63....	90	8	8.8	72...	173	25	14.4
			Av. percentage, 10.5				Av. percentage, 8.3

able I, *a*, *b* and *d* shows that the average percentage of larvae ted was constantly less from saturated than from moist soil. This is also shown in Table V and in Table II where it can be seen the percentage from saturated clay was uniformly low.

TABLE II.

PERCENTAGE OF RECOVERIES FROM DIFFERENT TYPES OF SOIL.

Type of appar-atus used.	Amt. of soil.	Types of soil.	Moist. cont. of soil.	Tempt. of soil.	Tempt. of water.	No. lar-vae put in.	No. larvae re-cov-ered.	Per-centage of re-cov-eries.
Large	1 pt.	Cl. Loam	Moist	Rm. Tempt.	115° F.	81	62	76.5
"	"	"	"	"	"	46	25	54.3
"	"	"	"	"	"	54	47	89.2
"	"	"	"	"	"	73	52	71.2
"	"	"	"	"	"	61	45	73.6
"	"	Sand	"	"	"	49	30	61.2
"	"	"	"	"	"	67	14	20.8
"	"	"	"	"	"	83	46	55.4
"	"	"	"	"	"	51	29	56.8
"	"	"	"	"	"	71	46	66.1
"	"	Cl. Sand	"	"	"	40	0	0
"	"	"	"	"	"	50	4	8
"	"	"	"	"	"	51	11	21.5
"	"	"	"	"	"	50	2	4
"	"	"	"	"	"	74	13	17.5
Small	2 oz.	Cl. Loam	"	85° F.	116° F.	100	80	80
"	"	"	"	"	"	91	81	89
"	"	Sand	"	"	"	136	130	95.5
"	"	"	"	"	"	143	119	83.2
Large	1 pt.	Cl. Loam	"	Rm. Tempt.	115° F.	150	128	85.3
"	"	"	"	"	"	146	81	55.4
"	½ pt.	Clay	Saturated	"	"	125	30	24
"	"	"	"	"	"	149	54	38
"	"	"	"	"	"	100	21	21
"	"	"	"	"	"	137	25	19
"	"	"	"	"	"	130	46	35
"	"	"	"	"	"	139	32	23
"	"	"	"	"	"	127	31	26
"	1 pt.	"	"	"	"	550	220	40
"	"	Humus	Moist	"	"	250	125	50

able II shows the effect of different types of soil on the per-ge of larvae isolated. Test numbers 1 to 15 are the results of an riment (by Augustine) to compare the percentage of recoveries in three different types of soil, treated in exactly the same way, and using larvae from the same culture. The clay loam was the same soil used in Table I, the sand was a coarse red sand and the clay sand was a very finely divided sand and clay mixture. The low percentage of recoveries from the clay sand indicates that when the particles of soil are very fine, so that they pack closely together, the escape of the larvae into the water is inhibited. Test numbers 10 to 19 of Table II also give a comparison of the recoveries from clay loam and sand obtained in a different series of experiments (by Augustine). The percentage of larvae isolated here is very high, perhaps because only 2 oz. of soil were used, but no differences can be noted between the clay loam and the sand, which in this case was also coarse. Test numbers 20 to 30 of Table II were controls used in the course of examinations from the field (by Cort). The low percentage of recoveries from saturated clay supports the view expressed in regard to the clay sand, because the particles of the clay were so finely divided that, when saturated, they became closely packed, reducing greatly the spaces in the soil. That the texture of the soil is an important factor in the percentage of larvae which can be isolated is shown clearly by the series of experiments (by Payne) in Table III. This table shows that the mixing of coarse gravel with soil increases very appreciably the percentage of larvae that can be isolated from it, and indicates that, when dealing with very finely divided soil in field samples, the mixing in of sterilized coarse soil or gravel would be advantageous

TABLE III.

THE EFFECT OF MIXING COARSE GRAVEL WITH SOIL ON THE PERCENTAGE OF LARVAE RECOVERED.

Type of soil.	Amount of soil.	Numbers of hookworm larvae put in.	Number recovered.	Percentage of recoveries.
Clay Loam packed into a solid ball	Ball 1½″ in diameter	20	4	20
Clay Loam packed into a solid ball	Ball 1½″ in diameter	47	5	10.6
Clay Loam packed into a solid ball, but mixed with gravel.	Ball 1½″ in diameter	59	55	93
Clay Loam packed into a solid ball, but mixed with gravel.	Ball 1½″ in diameter	39	39	100
Clay Loam mixed with gravel.	¼ pint	186	151	81

In Table IV are given the results of the isolation of larvae from a series of migration experiments (by Augustine). These tests differ from those previously recorded in that the larvae had lived in the soil for periods varying from 15 hours to 11 days before being placed in the apparatus. The higher percentage of larvae recovered in these experiments is probably due both to the use of smaller quantities of soil and to the fact that the larvae had a shorter distance to migrate. In some of these cases it was known that the hookworm larvae were on the surface, and, therefore, this part of the soil sample was put into the sieve first. This shows that in experimental work it may be possible to increase the percentage of larvae which can be isolated by careful handling of the soil.

TABLE IV.

PERCENTAGE OF RECOVERIES OF LARVAE WHICH HAD BEEN IN SOIL FROM 15 HRS. TO 11 DAYS. SOIL USED: MOIST CLAY LOAM. SOIL AT ROOM TEMPERATURE. WATER 118° F.

Test No.	Time larvae had lived in soil.	No. larvae put in soil.	No. larvae recov. with small appr.	No. larvae recov. with large appar.	Total No. larvae recov.	Percentage.
1....	15 hours	800	409	115	524	**66.7**
2....	15 “	1,000 (?)	583	275	858	**85.8**
3....	15 “	1,000 (?)	460	406	866	**86.6**
4....	15 “	525	363	69	432	**81.9**
5....	2 days	300	248		248	**82.6**
6....	2 “	300	289		289	**96.3**
7....	3 “	100	87		87	**87.0**
8....	4 “	300	217		217	**72.3**
9....	4 “	300	259		259	**86.3**
10....	6 “	100	52		52	**52.0**
11....	9 “	100	51		51	**51.0**
12....	11 “	512	89	148	237	**46.4**

The experiments shown in Table V (by Payne) give an idea of the length of time it takes for larvae to pass through the isolating apparatus. The data in this table show that, while the great majority of the larvae which can be recovered pass into the water within six hours, there is an appreciable number which can be recovered only after a longer period. It is significant to note that a larger percentage of the larvae come out of saturated soil between six and twenty-four hours than from the moist soil. It is possible that if the saturated soil were left for a still longer period, the percentage of larvae isolated would be increased.

TABLE V.

EXPERIMENT TO TEST THE TIME OF PASSAGE OF LARVAE THROUGH ISOLATING APPARATUS. SOIL: CLAY LOAM AT ROOM TEMP. WATER AT 115° F.

A. Soil moist.

Test No.	Type of apparatus.	Amt. of soil.	Larvae put in.	Larvae recovered after								Total No.	Percentage.
				1 hr.		3 hrs.		6 hrs.		24 hrs.			
				No.	%	No.	%	No.	%	No.	%		
1.....	large	1 pt.	140	12	8.6	34	24.2	17	12.1	6	4.3	69	49.2
2.....	small	½ pt.	113	29	25.7	35	30.9	21	18.6	5	4.4	90	79.6
3.....	"	¼ pt.	106	19	17.9	23	21.7	15	14.1	2	1.9	59	55.6
4.....	"	¼ pt.	83	24	28.9	36	43.4	10	12	3	3.6	73	87.9
B. Soil saturated.													
5.....	large	1 pt.	124	8	6.4	17	13.7	6	4.8	17	13.7	48	38.6
6.....	small	½ pt.	105	3	2.8	19	18.1	9	8.6	15	14.3	46	43.8
7.....	"	¼ pt.	111	1	0.9	15	13.5	6	5.4	17	15.3	39	35.1
8.....	"	¼ pt.	93	8	8.6	12	12.9	10	10.8	12	12.9	42	45.2

The series of experiments just described serves as a control on the examination of soil samples from the field where the number of larvae was unknown, gives some idea of the limitations of the isolating apparatus, and indicates in a general way the method by which it works. A number of points are still not clear. Just what causes the larvae to come out of the soil into the water is still unknown. If it were an attraction to moisture they would come out into water of about the same temperature as the soil as readily as into warmer water and would make their escape quickly, before the whole mass of the soil becomes uniformly moistened. That the escape is a direct reaction to the higher temperature of the water seems impossible, because in a very short time the whole soil in the sieve becomes of the same temperature as the water, and even after six hours a number of larvae will escape. Since the percentage of larvae which can be isolated is much greater in porous than in very compact soil, it is possible as suggested by one of us (Payne) that the larvae, stimulated to activity by the warmth and aided by the currents, escape by falling through the water-filled spaces of the soil. Be that as it may, the important point is that the apparatus really works, and makes it possible to isolate a considerable percentage of the larvae present, even from a rather large volume of soil.

USES OF THE ISOLATING APPARATUS IN INVESTIGATIONS AND HOOKWORM CONTROL WORK.

The apparatus which has just been described was utilized in every phase of the researches carried out in Trinidad during the past summer. In the field investigations its use made possible the examination of large numbers of soil samples from various situations, in areas where hookworm disease was prevalent, to determine the exact sources of infestation of the people. It also made it possible to follow the reduction of soil infectivity after the elimination of soil pollution. In the experimental work it was used in the study of the conditions under which the hookworm eggs can develop, and has made it possible to determine the extent of the migrations of the larvae in different situations. It was also utilized in studying the rate of unsheathing and the length of time the larvae can live in different soils. The uses made of this apparatus by Baermann and by our expedition suggest a wide range of possibilities in the study of hookworm larvae, both in the field and in laboratory experimentation. Further, it might be used in the study of free-living nematodes, and might be of practical help in investigations on the soil stages of nematodes parasitic on plants. The use of the isolating apparatus will also make possible advances in the knowledge of all parasitic nematodes which have a free-living stage, such as Strongyloides stercoralis and the Strongylidae of domesticated animals. Aside from the value of this apparatus in investigations, it can be successfully utilized, as shown by Baermann's work, in connection with campaigns for the control of hookworm disease, in locating and evaluating the sources of infestation of the people, and in discovering the sources of reinfestation after the completion of a piece of work.

SUMMARY.

1. The apparatus for isolating hookworm larvae from soil, described in this paper and shown in Figs. 1 and 2, which is similar to an apparatus devised by Baermann, makes it possible to isolate hookworm larvae from considerable quantities of soil.

2. It is possible to distinguish mature hookworm larvae, both sheathed and unsheathed, from other nematodes found in soil, by their characteristic structure and movement.

3. For experiments with the isolating apparatus mature hookworm larvae were obtained by spreading feces containing hookworm eggs on soil in large pans and covering this with a thin layer of ashes.

4. Experiments to standardize the isolating apparatus showed (1) that to isolate a satisfactory percentage of the larvae the water must be at least 10° F. warmer than the soil, (2) that a slightly higher percentage of larvae can be isolated from moist than saturated soil, (3) that in soil with very finely divided particles the percentage of larvae which can be isolated is less than in coarser soils, and (4) that while most of the larvae escape from the soil into the water, in the first six hours, an appreciable number come out after this time.

5. It is probable that the passage of the larvae from the soil to the water is not a reaction to moisture or a higher temperature, but is brought about by the active larvae falling through spaces in the soil until they reach the water.

6. The isolating apparatus makes possible the study of soil nematodes, both free living and the larvae of parasitic species, in their natural environment, and can be utilized in investigating the sources of human infestation in hookworm control work.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

III. A DISCUSSION OF THE FINDING OF UNSHEATHED HOOKWORM LARVAE IN THE SOIL.*

BY WILLIAM W. CORT, PH.D., DONALD L. AUGUSTINE, B.S., JAMES E. ACKERT, PH.D., FLORENCE KING PAYNE, M.D., AND GEORGE C. PAYNE, M.D.

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Looss (1911, p. 429) makes the following statement in his monograph on the hookworm: "During the rest of their free existence, the mature larvae are surrounded by the detached skin of the second larval stage, which acts as a protective envelope. It is thrown off in the normal course of events when the worms pass into a suitable host and thus enter the third stage of their life." These two sentences sum up the conception which dominates our present ideas of the life of infective hookworm larvae. In fact the terms "encysted," "encapsuled" or "sheathed" larvae are commonly used as synonyms of infective larvae. Accepting this conception, we were surprised to find that it is a common occurrence for mature hookworm larvae to loose their sheaths in soil, and that such unsheathed larvae are able to continue their lives and are probably infective. On account of the far-reaching significance of this finding and because it forms a part ot the data and conclusions of the investigations of all the members of our expedition, we decided to publish the present paper jointly.

The fact that infective hookworm larvae may become unsheathed under certain conditions was first noted by Leichtenstern (1887). Other authors who reported this phenomenon were Lambinet (1902), Herman (1905), and Looss (1911). As Looss (1911, pp. 429–436) has discussed in detail the findings of these authors, it will not be necessary to consider them further here. All of these invesigators were so influenced by the idea that the infective hookworm larvae live normally enclosed in sheaths that they considered their findings as representing abnormal conditions. More recently Baermann (1917, 624) noted the presence of unsheathed forms amongst larvae which he isolated from samples of soil. He apparently experienced difficulty in identifying such unsheathed larvae as young hookworms, and ascribed the loss of the sheath to the effect of the passage through the isolating apparatus, which he used.

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* This paper is the third of the series on the hookworm investigations by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

The first intimation that infective hookworm larvae might loose their sheaths in soil came early in our work, when one of us (Augustine) found in one of his experiments that some larvae which had been placed in the soil came to the surface without their sheaths. Following this discovery similar findings were made in quick succession in all of our experiments and in the samples from the field. The data given below, taken from our studies in Trinidad, show that a large percentage of hookworm larvae complete their second moult in the soil and live in the unsheathed condition.

In connection with some studies (by Cort and Payne) which will be included as later numbers of this series, a large number of samples of soil were examined for hookworm larvae. In a series of samples taken from a cacao grove, where concentrated centers of soil pollution occurred, the sheathed and unsheathed larvae of twenty-four samples were counted. Out of a total of 2,159 infective hookworm larvae isolated from these samples, 1,806 were sheathed and 353 unsheathed, or 83.6 per cent. sheathed and 16.4 per cent. unsheathed. The considerably higher percentage of sheathed larvae found in this series is partly explained by the findings from one soil sample, from which was obtained a total of 1,802 sheathed and 28 unsheathed hookworm larvae.

Much more striking were the findings from a large series of soil samples taken from a highly polluted area in a sugar cane field. In 108 positive soil samples from this area, the number of infective hookworm larvae per sample varied from 1 to 937. Out of a total of 4,265 hookworm larvae isolated from these samples, 1,793 were sheathed and 2,471 unsheathed or 42 per cent. sheathed and 58 per cent. unsheathed. These soil samples were taken from two types of localities, the first type at regular intervals along the drains, and the second between the rows of cane, at places where pollution was known to have occurred. Although stools were occasionally found in the drains, the samples were not taken at definite polluted spots as were those from between the rows, and they were therefore not so directly related to the points of pollution. Further the small numbers of larvae obtained from the drain samples and their almost uniform distribution along the drains suggested that these larvae were some distance from the point of development. With this in mind, it is interesting to make a comparison between the percentages of unsheathed larvae from these two situations. In the drains, in 39 positive samples, there were found 698 mature hookworm larvae of which 135 were sheathed and 563 unsheathed or 19.3 per cent. sheathed and 80.7 per cent. unsheathed. From the 69 positive samples taken from polluted spots between the rows 3,567 mature hookworm larvae were isolated. Of these 1,658 were sheathed and 1,909 unsheathed, or 46.5 per cent. sheathed and 53.5 per cent. unsheathed. The higher percentage of unsheathed larvae in the drains may perhaps be related to the fact noted above that the samples were not taken at centers of pollution, and therefore that larvae were probably farther away from the places where they developed, and perhaps on the average older than those taken from the other situation.

TABLE I.

Data on unsheathing of hookworm larvae in migration experiments.

No. of experiment.	Original number of sheat'd larvae.	Age of culture from which larvae were obtained.	Type of soil used.	Time that larvae remained in soil.	Number of sheat'd larvae recovered.	Number of unsheat'd larvae recovered.	Percentage of sheat'd larvae recovered.	Percentage of unsheat'd larvae recovered.
1....	1,000	5 days	Moist black clay loam	15 hrs.	164	702	18.9	81.1
2....	512	5 days	Moist black clay loam	11 days	3	234	1.2	98.8
3....	525	5–6 days	Moist red clay loam	15 hrs.	36	396	8.3	91.7
4....	1,300	8 days	Moist sand	7 days	43	332	11.4	88.6
5....	1,300	8 days	Saturated sand	8 days	57	800	6.6	93.4
6....	1,000	7 days	Moist black clay loam	7 days	180	141	56.0	44.0
7....	1,000	7 days	Moist black clay loam	7 days	245	272	47.3	52.7

In connection with a series of experiments on the migration of mature hookworm larvae in various types of soil (by Augustine) some interesting data were obtained on the loss of the sheath. In these experiments sheathed larvae, five to eight days old, were placed in an area one inch in diameter at the center of the surface of soil which was spread out to the depth of 1.5 cm. in thirteen-inch pans.

This soil was later divided into zones and placed in the isolating apparatus, for the removal of the larvae. The complete results of these and similar experiments on migration will be given in a later number of this series. In the present paper we are interested in the fact that young sheathed larvae were placed in these pans, and after periods of time varying from 15 hours to 11 days a high percentage of unsheathed larvae was recovered. Table I gives a summary of these results.

These experiments were performed in the laboratory, the surface of the soil in the pans being sprinkled every day. It will be seen from Table I that a high percentage of unsheathed larvae was obtained, even when the matured hookworm larvae had been in the soil only 15 hours. Since different types of soil were used this series indicates that the loss of the sheath does not depend on any particular type of soil. In connection with these experiments microscopic examinations were made directly of small particles of soil from the centers, where the larvae had been placed. On one piece of clay loam two active unsheathed hookworm larvae were found and one empty sheath, which had its anterior part imbedded in soil. Another direct examination was made of a small particle of sand. On this were found six active unsheathed larvae and three empty sheaths.

Another series of experiments was carried out by one of us (Ackert), giving further evidence on the unsheathing of the mature hookworm larvae. These experiments which will be described fully in a later number of this series were undertaken to study the conditions under which hookworm eggs will hatch and develop. Portions of fresh stools from individuals heavily infested with hookworms were placed in soil in flower pots. These flower pots were then placed out of doors, where they were subjected both to sun and shade, and wetting from rains followed by partial drying. Table II includes the data from these experiments which relate to the unsheathing of the larvae.

The conditions in these experiments are more like those in nature than in the former series, since in every case feces containing hookworm eggs were used. The large number of unsheathed larvae which were isolated add to the evidence that the loss of the sheath is a common phenomenon. A comparison of the experiments numbered XXVI, XXVII, XXVIII and XXIX is very interesting. In these experiments the conditions were the same, except that the last two were kept four days longer than the others. The additional time was

TABLE II.

Data on percentage of sheathed and ***unsheathed*** *larvae found in series of flower pot experiments.*

No.	Soil.	Conditions of experiment.	Conditions of light.	Days out.	Sheathed.	Un-sheathed.	Percentage sheathed.	Percentage un-sheathed.
I . .	Clay loam	Buried ½ inch	Sun and shade	29 days	0	33	0.0	100.0
V . .	Clay loam	Buried ½ inch	Shade and sun	21 days	52	30	63.4	36.6
XVI . .	On clay loam	Covered 8 mm.: with ashes	Shade and sun	23 days	420	885	32.1	67.9
XVIII . .	Clay loam	Buried ½ inch	Shade	25 days	1,861	0	100.0	0
XIX . .	Clay loam	Buried ½ inch	Shade and sun	27 days	139	30	82.2	17.8
XXVI . .	Clay loam	On top	Shade and sun	13 days	3,250	0	100.0	0
XXVII . .	Clay	On top	Shade and sun	13 days	1,112	0	100.0	0
XXVIII . .	Clay loam and clay	On top	Shade and sun	17 days	53	400	11.2	88.8
XXIX . .	Clay loam	On top	Shade and sun	17 days	145	1,564	8.4	91.6
XXX . .	Clay loam	On top	Light and shade	18 days	13	119	9.8	90.2
XXXV . .	On clay loam	Covered 3 mm.: in ashes	Shade	23 days	2,250	720	75.5	24.5
XXXVI . .	Ashes	Buried 3 mm.	Shade	23 days	525	508	50.8	49.2
XXXVII . .	On clay loam	Buried 8 mm.	Shade	23 days	2,469	0	100.0	0
XL . .	On clay loam	Buried 8 mm. in ashes	Sun and shade	15 days	108	161	40.1	59.9
XLI . .	Clay loam	On surface	Sun and shade	15 days	10	1,290	.7	99.3
XLII . .	Clay loam	Buried ½ inch	Sun and shade	14 days	1,304	971	57.3	42.7

apparently the factor which caused a very rapid unsheathing. A further analysis of the table shows that no difference can be found in the percentages of unsheathed larvae between those experiments in which the fecal material was placed on the surface of the soil and those in which it was slightly buried. As far as the data go they seem to indicate that when the flower pots were exposed to alternating sunlight and shade there was a greater degree of unsheathing than when they were kept in the shade. A comparison of experiments XVIII, XXXV, XXXVI and XXXVII, which were kept in the shade, with others, in which the conditions were the same except for alternating sunlight and shade supports this view. Perhaps the alternating sunlight and shade with the partial drying of the soil stimulated the larvae to activities, which resulted in the greater degree of unsheathing.

In a series of experiments performed by one of us (Florence King Payne), to test the distance which hookworm larvae can migrate, when buried in soil, additional data were obtained on the unsheathing of the larvae. Sheathed larvae were placed at the bottom of columns of soil of various heights, and later this soil was divided into zones, placed in the isolating apparatus, and the hookworm larvae obtained counted. The soil was a black clay loam. Table III shows the percentage of sheathed and unsheathed larvae recovered in this series of experiments.

It is shown in Table III that when sheathed larvae are buried in soil for periods varying from two to ten days, a considerable percentage of them lose their sheaths. Further, under the conditions of these experiments the percentage of unsheathed larvae increased the

TABLE III.

Findings of hookworm larvae from soil in vertical migration experiments.

No. of days larvae had remained in soil.	Total number recovered.	Number sheathed.	Number unsheathed.	Percentage sheathed.	Percentage unsheathed.
2 days........	256	129	127	50.4	49.6
3 days........	26	12	14	46.0	54.0
4 days........	1,505	767	738	50.8	49.2
6 days........	14	4	10	28.5	71.5
7 days........	418	126	292	30.1	69.9
8 days........	271	56	215	20.6	79.4
9 days........	34	17	17	50.0	50.0
10 days........	30	3	27	10.0	90.0

longer the larvae remained in the soil. Another important point was shown in one of the experiments when, after four days, 188 larvae were recovered which had migrated vertically between 5 and 6 inches. Of these larvae 140 were sheathed and 48 unsheathed, or 74.5 per cent. sheathed and 25.5 per cent. unsheathed. This observation is significant since it shows that the larvae do not necessarily lose their sheaths while migrating through clay loam soil.

The data given above show that under the conditions in Trinidad, during the months of June, July and August, when the daily range of temperature is from about 74° to 94° F., infective hookworm larvae very commonly lose their sheaths in soil and continue to live actively.

It is a well-known fact that the mature hookworm larvae are infective while still enclosed in their sheaths, but that for penetration into their host they must break out of their sheaths leaving them on the surface of the skin. It would seem, therefore, that active larvae already unsheathed would be able to penetrate the skin more easily and quickly than those which come in contact with the surface of the skin while still enclosed in their sheaths. That unsheathed mature hookworm larvae can penetrate mammalian skin was shown by one of us (Augustine) in an experiment in which unsheathed larvae were observed to penetrate the skin of a rat.

The cause of the loss of sheath and the exact conditions under which it occurs are not clear from the data at hand. Baermann's idea, that the finding of unsheathed hookworm larvae in his examinations was due to the passage through the isolating apparatus, cannot be applied to our findings. We have often found in our controls that some unsheathing does occur in the passage through the soil in the sieves, but in most cases practically all of the larvae will pass through the apparatus without losing their sheaths. It has already been noted that unsheathed larvae were found by direct microscopical examinations of small particles of soil. Further, soil samples taken from the cane-field and treated in exactly the same way show all degrees of variation in the percentage of sheathed and unsheathed larvae, after passage through the isolating apparatus. One sample yielded 1 sheathed and 247 unsheathed, another gave 407 sheathed and no unsheathed, while other samples showed all gradations between these extremes.

Few observations have been made by us which bear on the cause of the loss of the sheath, and the conditions under which this loss occurs. That the unsheathing is not limited to any particular type of soil is shown by the fact that it occurs in clay, clay loam and sand. It also occurs in differing conditions of moisture. That a longer time in the soil seems in some cases to increase the percentage of unsheathed larvae is indicated by some of our experiments, and yet in other cases a very high percentage of unsheathed larvae was obtained after only 15 hours in the soil, and in another experiment 25 days after feces containing eggs had been placed in soil large numbers of sheathed, but no unsheathed larvae were isolated. Certain experiments show that the unsheathing is greater where the soil containing the larvae is exposed to alternating sunlight and shade, than when it is kept constantly in the shade. This might indicate that conditions which increase the activity of the hookworm larvae in the soil tend to bring about the loss of the sheath, but the fact already cited, that larvae which had migrated vertically through 5 to 6 inches of clay loam remained sheathed, shows that they can go through a period of intense activity completely surrounded by soil, and still retain their sheaths. Perhaps further investigations on the rate and degree of unsheathing under different conditions of soil, temperature and moisture will give an explanation of the cause of the unsheathing of the infective hookworm larvae in soil.

As stated earlier in this paper, the finding that the loss of the sheath is a common process in infective hookworm larvae in the soil necessitates a revision of many ideas in regard to this phase of the life-history of the hookworm. Most of the knowledge of the activities of the infective hookworm larvae has come from experiments on sheathed larvae, studied under unnatural conditions, such as in cultures, in water, or before they have left the fecal mass, where they develop. Of what actually occurs in soil little is known, and since it has now been discovered that a very large percentage of hookworm larvae lives in the soil without any protective sheath, it would seem necessary to make investigations of various kinds on the activities and relation to environment of the unsheathed larvae, and also on the larvae as they live in the soil under natural conditions. Such investigations on a comparatively large scale are made possible through the use of the isolating apparatus described in the second number of this series (Cort et al., 1921). That studies of this kind will give some results quite at variance with present ideas is shown by the results of the experiments carried out in Trinidad, which will be given in the succeeding numbers of this series.

SUMMARY.

1. Although various investigators have reported that mature hookworm larvae lose their sheaths under certain conditions, it has been the general opinion that they live normally enclosed in sheaths.

2. It was found that in a series of soil samples examined from places polluted by people infested with hookworms a large proportion of the mature larvae were without sheath.

3. Also, in experiments on the conditions under which hookworm eggs hatch and develop and on the migrations of the infective larvae, it was found that in soil a large proportion of the larvae soon became unsheathed.

4. These findings suggest that it is a common thing for mature hookworm larvae to lose their sheaths and continue to live in soil.

5. Since most of the knowledge of the activities of mature hookworm larvae has come from studies on sheathed forms, it will probably be necessary to revise many of our ideas on the life of infective hookworm larvae in the soil.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

IV. THE RELATION OF THE DOMESTIC CHICKEN TO THE SPREAD OF HOOKWORM DISEASE.*

BY JAMES E. ACKERT, PH.D.

(Received for publication, September 20, 1921.)

INTRODUCTION.

It has long been known that domestic chickens will disseminate human excrement, and as early as 1910 Dock and Bass (p. 87) ascertained that hookworm eggs can retain their viability and hatch after passing through a chicken's digestive tract. But the proportion of hookworm eggs which produce infective larvae seems to be wholly unknown. As chickens usually abound in regions where hookworm disease is prevalent, it is important to determine whether they are a real factor in the spread of the disease. An opportunity to investigate this problem in Trinidad, British West Indies, where over eighty per cent. of the natives are infested with hookworm, was recently afforded me by the International Health Board, through the efforts of Dr. William W. Cort and Dr. George C. Payne. I wish at this time to acknowledge my indebtedness to them for complete cooperation and many helpful suggestions, and also to my other colleagues, Dr. Florence King Payne, and Mr. D. L. Augustine.

METHODS.

For this and other experiments a private dwelling was leased and converted into a field laboratory. As the house was built on piles, very suitable animal quarters were afforded underneath it. The investigations were begun at the close of the dry season, and the clay-loam soil beneath it was found to be wholly free from hookworm larvae and nearly so from free-living nematodes. The experiments were facilitated by the absence from the premises of children and other irresponsible persons who might pollute the soil.

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* This paper is the fourth of the series on the hookworm investigations made by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, with the Cooperation of the International Health Board, Rockefeller Foundation. It is Contribution No. 55 from the Department of Zoology, Agricultural Experiment Station, Kansas State Agricultural College.

For the chicken experiments two pens were constructed, a large living pen and a small one for the recovery of feces. The small pen was equipped with a woven wire platform that permitted the feces to drop upon clean paper. To ascertain the length of time required for material to pass through the chicken's digestive tract two filter paper capsules of animal charcoal and feces were given to each fowl. In two hours and forty minutes the first black fecal deposit appeared, and for several hours all droppings showed the animal charcoal. At the end of twenty hours after the feeding, the feces were grayish, showing no trace of the animal charcoal. Consequently, in the quantitative tests the feces were usually recovered for at least twenty hours after the feedings were made.

As chickens carry human excrement on their feet, this source of error in the experiments was avoided by administering the feces containing hookworm eggs to the chickens in filter paper capsules. Thus any hookworm larvae found in the pens could have come only from eggs that had passed through the digestive tracts of the fowls. The recovered chicken feces containing hookworm eggs were cultured in animal charcoal in closed Petri dishes and soup plates, and in previously heated garden soil in open pans, both in the laboratory and out of doors. The garden soil as a culture medium worked very satisfactorily both for human and for chicken feces. The gradual heating up to 150° F. did not oxidize the organic material, but killed all nematodes, both sheathed and unsheathed. The feces containing hookworm eggs were placed on top of such soil, or scratched into the surface, approximating natural conditions. After allowing six to ten days for the hookworm larvae to reach the infective stage the cultures were placed in separate units of the isolation apparatus (Cort et al., 1921) and the hookworm larvae obtained counted. The first tests were made with two adult chickens. Their feces voided over night and examined both by smears and the brine-loop-flotation technique showed no nematode eggs or larvae. The various findings were verified by the use of two young chicks about six weeks old. Their feces likewise were free from nematode eggs and larvae.

CAN HOOKWORM EGGS SWALLOWED BY CHICKENS PRODUCE INFECTIVE LARVAE?

A preliminary series of experiments confirmed the early work of Dock and Bass already cited, viz.: that hookworm eggs can retain their viability and hatch after passing through the digestive tract of the chicken. However, the critical test of the chicken as a factor in the spread of hookworm disease is not whether hookworm eggs can remain viable in passing through its alimentary canal and then hatch, but whether these larvae can develop to the infective stage.

To decide this point, a series of feedings was made, the essential features of which are shown in Table I. For this and subsequent feedings the stools of one individual, known as Beggar No. 2, were utilized because of the uniformly large number of hookworm eggs present and the comparative lack of other nematode eggs and larvae. The "cubic inches" of stool administered are approximate rather than accurate, nine filter paper capsules being about equal to one cubic inch. Only sufficient animal charcoal was mixed with the fresh stool to darken it. After recovery the chicken feces were mixed with an equal amount of animal charcoal and cultured in closed Petri dishes and covered soup plates. As shown in Table I, the subsequent examinations revealed infective hookworm larvae varying in the different cultures from one to forty-six.

TABLE I.

Development of infective hookworm larvae in chicken feces and animal charcoal.

Date.	Cubic inches of stool fed.	Feces recovered hrs. after feeding.	Culture medium.	Days cultured.	No. infective larvae recovered.
May 21st....	2	29	Animal charcoal	12	13
May 30th....	1½	22	Animal charcoal	12–15	46
June 1st.....	½	5½	Animal charcoal	10–15	13
June 11th...	¾	5½	Animal charcoal	11–25	1

As animal charcoal is known to inhibit putrefaction of the stool it was thought that the larvae might be able to develop to the infective stage in a culture medium of fowl feces and charcoal and not be able to do so in chicken feces and soil. This, however, proved not to be true, as seven sheathed hookworm larvae were obtained from a culture of fowl excrement and previously heated soil.

Again, it might happen that larvae could mature in such a medium in closed Petri dishes, but fail to do so out of doors in the absence of closed containers kept in the shade. But this doubt was likewise removed when, after a feeding, sixty infective hookworm larvae were taken from a culture of chicken feces and previously heated soil which was placed out of doors in the sun and rain.

WHAT PROPORTION OF THE HOOKWORM EGGS SWALLOWED BY CHICKENS PRODUCE INFECTIVE LARVAE?

An examination of Table I and of the two tests just described reveals the fact that in none of the cultures of chicken feces has a large number of infective hookworm larvae developed. This observation led to an attempt to make some quantitative tests of the proportionate number of larvae developing to the infective stage in such cultures. In these tests the fresh stools of Beggar No. 2 were divided into two very nearly equal portions, one half being cultured directly and the other half given to the chickens. After several hours the chicken feces containing the hookworm eggs were recovered, cultured, and in all respects kept under conditions similar to those of the corresponding human culture. These conditions included culturing in Petri dishes and in earthen pans in the laboratory, and in open containers out of doors. In Table II are given the principal data obtained in the tests. The finding of only one hookworm larva in the test of June 11 might be explained by the fact that the chicken feces were recovered for only 5 hours and a half after feeding, although before the end of this time all of the blackened part of the feces had been voided. The feces from the June 12 test were mixed with previously heated soil and placed in eleven-inch tin pans whose bottoms had been perforated for drainage. These cultures were kept in the laboratory except during the almost daily shower, when they were set out until thoroughly soaked. Thus they were in the sun but a few minutes at a time, immediately after showers. As no animal charcoal was used in administering the feces, and ample time was allowed for the passage of the hookworm eggs through the chickens, this seems to have been a very fair test.

The test of June 18 was similar to the one just described, except that the feces in each case were slightly scratched into the surface of the soil and the cultures set out of doors, where they received approximately four hours of sunshine and a heavy or light shower almost

TABLE II.

Effect of chicken on hookworm development.

Date.	Cubic inches of stools.	Disposal of stool.	Feces recovered hrs. after feed.	Culture medium.	Environment.	Days cultured.	Infective hookworm larvae obtained.	Percent. of larvae from chickens.
June 11..	1½	Half cultured		Animal charcoal	Laboratory	11	139	
		Half to chickens	5½	Animal charcoal	Laboratory	11-25	1	.7
June 12..	3	Half cultured		Heated soil	Shade	11	4,780	
		Half to chickens	17	Heated soil	Shade	10	215	4.2
June 18..	2	Half cultured		Heated soil	Shade	9	1,744	
		Half to chickens	23	Heated soil	Shade	8	58	3.3
June 30..	3	Half cultured		Heated soil	Sun and shade	9	2,802	
		Half to chickens	22	Heated soil	Sun and shade	8	88 (264)	9.4
July 18..	3	Half cultured		Heated soil	Shade	13	3,180	
		Half to chickens	21	Heated soil	Shade	12	9	.3
July 28..	2	Half cultured		Heated soil	Shade	9	882	
		Half to chickens	25	Heated soil	Shade	8	138	15.6
Aug. 4..	2	Half cultured		Heated soil	Shade	9	925	
		Half to chickens	23	Heated soil	Shade	8	54	5.8

daily. This test represented a close approximation of natural conditions. The next test, June 30, differed slightly in that the feces were simply placed on top of the previously heated soil and then set out of doors. The combined action of fly larvae and rain caused a complete disappearance of the human portion during the third day, while the more fibrous fowl feces were in evidence throughout the eight-day period. In recovering the infective larvae from the chicken culture, the centrifuge tube containing the larvae was emptied after about one third of the fluid was examined. However, as the fluid and larvae had been thoroughly mixed just before the first third was withdrawn for examination, it is probable that the 88 larvae counted represent approximately one third of the number 264.

In the tests of July 18 and 28 extreme care was exercised in making an equal division of the human stool. The feces were placed on top of the culture soil, but the pans were kept in a small closed yard house and sprinkled frequently, avoiding the heavy rains and the direct rays of the sun. But these precautions did not raise the percentage of infective hookworm larvae developing in the chicken feces. Immediately before and after the feeding of August 4, four earthworms and some whole sweet milk were given to one of the chickens to ascertain if a higher protein diet might affect the number of hookworm larvae developing to the infective stage. However, it is seen (Table II) that this had no appreciable effect, the number maturing being 5.8 per cent. of the number attaining the infective stage in the corresponding human culture. These tests indicate that hookworm eggs swallowed by chickens are much less likely to produce infective hookworm larvae than are the eggs in human stools deposited out of doors. As a whole, the tests indicate that less than ten per cent. of the eggs ingested by chickens produce mature hookworm larvae.

THEORETICAL CONSIDERATIONS.

The results from the tests shown in Table II must not be considered exact as several possible sources of error are involved. In the first place, the division of the stool into halves was made optically. But a difference in volume of five per cent. can readily be detected, so that the error here was certainly small. Next, the eggs may not have been evenly distributed through the stool. The only checks on this point were the frequent microscopic examinations of Beggar No. 2's stool, every smear of which showed hookworm eggs. In the third place, any eggs that happened to pass into the caeca on their way through the intestine would probably be delayed too long to be recovered. But this number would not be large. Then, of course, there is the possible error in counting. But after allowing a wide margin for these possible sources of error, it is evident that the great majority of hookworm eggs swallowed by chickens fail to produce infective hookworm larvae.

The cause of this phenomenon has not been definitely determined. However, a possible factor in the reduction is the action of the gizzard on the eggs. This seemed plausible when the writer learned of an experience Dr. Florence King Payne had with *Ascaris* eggs. In an attempt to separate the flocculent albumen from the eggs, small glass beads, two millimeters in diameter, were agitated in the culture dish, with the result that some of the eggs were broken. When eggs with shells as thick as those of *Ascaris* are broken by the concussion resulting from the shaking of small glass beads, it is readily conceivable that the thin-shelled hookworm eggs might suffer much injury in passing through the grit-filled muscular gizzard of the chicken. Especially might eggs be crushed that got into positions where the grit was fine and the muscular contractions severe. The only direct evidence on this point in the experiments was afforded by the difficulty of finding hookworm eggs in the chicken feces. When eggs were readily found in smears of the blackened human stool, it was surprising when six smears of the black chicken feces were examined without finding a single hookworm egg, although infective larvae developed in the culture. In view of these considerations, it seems possible that a part of the reduction in mature hookworm larvae occurring in the cultures of chicken feces and soil may result from injury to the hookworm eggs in the fowl's gizzard.

The inclusion of the urine with the chicken feces probably introduced an unfavorable factor in the production of larvae, as Minagawa (1917) found that the hatching of the hookworm eggs was greatly inhibited in latrines where the urine was mixed with the human excrement. In this author's opinion the failure of the eggs to hatch was due to differences in osmotic pressure caused by the presence of urine in the feces. To obviate this possible factor in the present experiments, the cultures from the tests of June 12, 18, and 30 were placed out of doors, where they were thoroughly washed by the rains nearly every day. The numbers of larvae maturing in these chicken cultures were larger, on the average, than those from such cultures kept indoors and lightly sprinkled, as was done with the cultures from the last three tests (Table II). The evidence here favors the urine hypothesis, but the eggs which produced the 138 larvae from the July 28 test probably hatched in the presence of more urine than did those which gave the 58 larvae from the June 18 test, as the former culture was only gently moistened by the aid of a pipette. However, in either case, it is probable that enough of the urine remained to affect the hatching of some of the eggs.

As the experimental chickens were kept under conditions which greatly reduced the amount of insect food, it might appear that their somewhat limited diet was responsible for the small numbers of hookworm larvae maturing in the feces, but the percentage of infective larvae did not increase when earthworms and milk were added to the rations which included corn, oats, and green grass.

A possible reducing factor may result from certain physiological and morphological differences between human beings and fowls. The greater avian oxygenation, higher temperature and different digestive apparatus, including a cloaca, may form a mixture of metabolic and egestible products such as to furnish rather unsuitable food for the developing of hookworm larvae. Other possible factors will doubtless be found upon further investigation. From the present data it seems probable that the reduction of the numbers of mature hookworm larvae resulting from the ingestion by chickens of hookworm eggs is due to three factors, grinding in the gizzard, differences in osmotic pressure caused by urine in the excrement, and malnutrition of the larvae in the feces.

THE ESTABLISHMENT OF INFECTIVE SPOTS BY CHICKENS.

While the number of infective hookworm larvae which could develop in a chicken's feces in one day is comparatively small, if this number were added day after day at the same place a dangerous infective spot would develop. Such a condition might arise around a drinking fountain or other wet place, where chickens are accustomed to come many times daily. To test this point, several samples of soil in the large chicken pen were again examined for hookworm larvae and found to be negative. The chickens were then given two feedings of Beggar No. 2's stool in the usual way, and the drinking fountain placed at one side of the pen on a spot also moistened by the showers. On the following day, several separate fecal deposits were seen near the drinking fountain, partially crushed into the moist soil. The moist condition continued and in two weeks a half-pint of soil taken beside the drinking dish yielded, through the isolation apparatus, seven infective hookworm larvae.

This finding was verified by testing a sheltered, dry spot several feet away, finding it free from hookworm larvae, moving the drinking fountain to it and making feedings as above. The next day a few fecal deposits were noted near the dish. The ground here was moistened by the splashing of chickens and an occasional wetting to imitate the rain. In a week, a half-pint of soil from this new place gave, through the isolation apparatus, nine infective hookworm larvae.

These positive tests suggested the possibility of an infective spot developing under the roost. Accordingly, a corner of the pen was tested for hookworm larvae and found not to contain them. The roosting frame was then moved into the corner and feedings of the hookworm eggs were made on July 6 and 18. As this corner was sheltered, the accumulating feces were moistened every few days. This mass was largely feces, but a small amount of dirt was scratched into it by the chickens. Two weeks after the last feeding, a pint of the mass was placed in the isolation apparatus, with the result that 157 infective hookworm larvae were counted, 107 being sheathed and 50 unsheathed. These experiments show that chickens swallowing human excrement containing hookworm eggs are able to establish infective spots on moist ground. In the rainy season in Trinidad such spots are likely to develop in any place which regularly attracts the chicken, such as the drinking fountain, the feeding ground (if they be fed) and the roosting place.

CAN NEWLY HATCHED LARVAE PASS UNINJURED THROUGH THE DIGESTIVE TRACT OF CHICKENS?

As hookworm eggs in warm, moist conditions are known to hatch in from 24 to 36 hours, and as chickens may thus swallow young unsheathed larvae, it is important to learn whether such larvae can pass through the digestive tract of the chicken uninjured. A portion of a four-day culture of human feces and animal charcoal containing many unsheathed hookworm larvae was administered to the chickens, when they had not been given feces for several days previously. Three hours after feeding, some of the freshly defecated black feces were examined by smear and five young unsheathed larvae were found in a small portion of it. The feces passed overnight were collected the following morning and thirteen smears taken at random were examined with the result that nine were positive, containing extremes of one and ten active hookworm larvae, making a total of twenty-four. This shows that the unsheathed hookworm larvae can retain their viability while passing through the digestive tract of the chickens. Unfortunately, no test was made to ascertain whether such larvae were able to develop to the infective stage. That they came through active and apparently normal makes it probable that they could reach the infective stage. This hypothesis is strengthened by the fact that newly hatched larvae of another species (*Strongyloides,* cited elsewhere), ingested by the chickens, were able subsequently to develop to the infective stage.

INGESTION OF INFECTIVE HOOKWORM LARVAE BY CHICKENS.

As chickens, in feeding about the yards and edges of the fields, are likely to swallow material containing infective hookworm larvae and later deposit it about the door yard, it seemed important to ascertain whether such larvae could be transported in this way. Four feedings of sheathed hookworm larvae were made, beginning on May 28, when 548 larvae were administered in filter paper and animal charcoal. All of the feces voided during the next 24 hours were collected, but examinations by smears and sedimentation failed to reveal an infective larva. A similar test was made on June 3d, when 500 infective larvae were administered in the same way. Examinations of the feces collected for 21 hours afterwards failed to yield a single hookworm larva.

A third feeding on June 9th was administered with a long pipette, the fluid which contained about 3,500 infective hookworm larvae being darkened with animal charcoal. All of the feces passed by these fowls during the next 22 hours were broken up and placed in a small isolation apparatus. Not a single hookworm larva appeared, whereupon the feces were sedimented, the residue being examined in Syracuse watch glasses and Petri dishes. Still no larvae could be found. The fourth feeding, including 3,400 infective larvae, was made four days later by the aid of a long pipette. In other respects the test was similar to the third just described. When the feces were placed in the small isolation apparatus, five mature hookworm larvae which had lost their sheaths were recovered. Further examination, by sedimentation and centrifugation, failed to yield any more larvae. These tests show that when infective hookworm larvae are swallowed by chickens they do not pass out at once unaltered. They may be digested or they may penetrate the wall of the alimentary canal. That all are digested is highly improbable, since young unsheathed larvae are known to survive passage through the chicken's food tube. Hence, it may be assumed that some of them enter the wall of the digestive tract. How long they remain in the chicken's body is problematical. Any that subsequently leave its body alive have probably so metamorphosed as to be unable to actively enter the body of a final host. That they do not continue development in the intestine of the chicken was determined when the two chickens used in these experiments were examined 67 days after the first feeding. A careful search on the walls of the intestines and through the entire intestinal contents failed to reveal a single hookworm larva. The results of these feedings show that the chicken rarely disseminates infective hookworm larvae. In fact they indicate that in swallowing such larvae the chicken may be a positive factor in hookworm control.

PRACTICAL SIGNIFICANCE.

The results of the various experiments show that in hookworm control the chicken is both beneficial and harmful. From the series of quantitative tests (Table II) it is evident that the great majority of the hookworm eggs swallowed by chickens fail to produce infective hookworm larvae. This is significant, for during the long rainy season in Trinidad a large percentage of the eggs in human stools, deposited out of doors, produce infective hookworm larvae, even though the stools be exposed to the direct rays of the sun during four or five hours daily. (Data on this point will be given by the writer, in this JOURNAL, in a later article of the series.) In such stools thousands of infective hookworm larvae develop in five days, whereas of the portions swallowed by chickens, only a small percentage of the eggs produce mature hookworm larvae. The scarcity of cereals and other ordinary chicken feeds on this island makes it imperative for the fowls largely to take what they can get, so that stools deposited where there are chickens usually disappear in a few hours. In this respect chickens are undoubtedly important positive factors in the control of hookworm disease during the rainy season.

On the other hand, hookworm eggs in stools, deposited out of doors during the dry season, probably all perish when left exposed, but of those eggs swallowed by chickens and dropped at the drinking place, a small percentage would produce infective hookworm larvae. Further, eggs in the fecal particles adhering to the feet of the chickens may also be brought to the drinking place where there is sufficient moisture to protect the developing hookworm larvae. Another detrimental phase of the chicken relationship that may occur either in the wet or dry season is the bringing of hookworm eggs by chickens from somewhat remote places up to the drinking or feeding spot. These spots, usually near the house, are repeatedly traversed by members of the family, most if not all of whom go barefooted.

Taking into consideration all of the *pros* and *cons* in the case, the writer is of the opinion that the great reduction of infective hookworm larvae resulting from the ingestion of quantities of human excrement by chickens outweighs the harm done by them in carrying eggs in their digestive tracts or on their feet to the door yard where infective spots may be established. In other words, the chicken does more good than harm. But in unsanitated districts or places where only certain members of the family use the latrine, the chickens are able to establish infective spots which in the door yard would be constant sources of danger.

STRONGYLOIDES STERCORALIS IN CHICKEN FECES.

Besides hookworm eggs in the stools used in the experiments, the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were of occasional occurrence, and a few newly hatched larvae of *Strongyloides stercoralis* were seen in the smears. These young larvae were able to survive passage through the chicken's body. No special studies were made to determine the minimum number of days required for them to develop to the infective stage in a culture medium of chicken feces and animal charcoal, or soil, but by 7 days after their passage through the chickens, fairly large numbers of them had developed the characteristic long filariform oesophagus, and were rapidly wiggling their way through the cultures. A single active mature male of the free-living generation was seen in a ten-day-old culture.

SUMMARY.

1. The length of time required for food material to pass through the digestive tract of chickens ranges from two hours and forty minutes to sixteen or more hours.

2. Hookworm eggs remain viable while passing through the alimentary canal of chickens and are able to hatch.

3. Hookworm eggs swallowed by chickens produce infective hookworm larvae when the fowl feces are mixed with animal charcoal or with soil.

4. The results of a series of experimental feedings indicate that

the great majority of hookworm eggs ingested by chickens fail to produce infective hookworm larvae. This failure is attributed in part to breaking of eggs in the gizzard, injury from urine in the feces, and to malnutrition of the larvae in the excrement.

5. Dangerous infective spots may be established around drinking receptacles by chickens that have swallowed hookworm eggs day after day.

6. Hookworm eggs deposited out of doors under conditions unfavorable for development can be carried by chickens to favorable environments.

7. Human stools voided in unfrequented places can be transported by chickens to the door yard which is traversed by bare-footed persons.

8. Newly hatched hookworm larvae can pass through the digestive tracts of chickens apparently uninjured.

9. Sheathed hookworm larvae swallowed by chickens fail to pass at once through the fowls unaltered.

10. *Strongyloides stercoralis* larvae occurring in the human stools passed through the chickens and soon attained the infective stage, a single mature male of the free-living generation having been seen.

11. The various experiments first performed on adult chickens were later repeated with young ones with similar results in each case.

12. A comparison of the reduction of mature hookworm larvae by the fowls with the establishment of infective spots by them convinces the writer that chickens are more beneficial than harmful in the control of hookworm disease in Trinidad.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

V. The Domestic Pig and Hookworm Dissemination.*

By JAMES E. ACKERT, Ph.D., and FLORENCE KING PAYNE, M.D.

(Received for publication, October 24, 1921.)

Introduction.

The free range frequently allotted the domestic pig, and its omnivorous habit have led to the suspicion that swine may be factors in hookworm dissemination. Experiments conducted by the Philippine Bureau of Science in 1913 indicated that the domestic pig could not be infected with human hookworms. Whether or not pigs may act as vectors in the dissemination of human hookworm eggs was not considered by them. In the absence of any record of experiments, and in order to investigate this point, the writers took advantage of an opportunity afforded in Trinidad, British West Indies, by the International Health Board of the Rockefeller Foundation, through the efforts of Dr. W. W. Cort and Dr. George C. Payne, to whom acknowledgment is here made for valuable suggestions.

Methods.

For the experiments three pens were used, a living pen, an experimental feeding pen and a small enclosure used only for collecting feces from experimental feedings. These pens, which were sheltered by the laboratory (Ackert, 1922), were free from hookworm larvae.

Two young pigs, approximately six weeks old, were used for the feeding experiments. Examinations of their feces by smears, sedimentation and centrifugation, and by the brine-loop-flotation technique failed to reveal the presence of any nematode eggs or larvae. By administering animal charcoal with food it was ascertained that sixteen hours were required for material to pass through the pigs. The human stools, which were mixed with corn meal and milk, and sometimes animal charcoal, were administered in the experimental feeding pen, care being exercised to avoid polluting the soil. The pigs were then placed in the small recovery room and the feces collected during the following twenty to twenty-fours hours. Upon recovery their feces containing hookworm eggs were cultured in animal charcoal or in garden soil which had been sufficiently heated to kill any hookworm eggs or larvae, but not hot enough to oxidize organic material. After a week or so the cultures in garden soil were placed in separate units of the isolation apparatus (Cort et al., 1922) which aided in isolating the infective larvae.

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* This paper is the fifth of the series of the hookworm investigations made by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University with the Cooperation of the International Health Board, Rockefeller Foundation. It is Contribution No. 56 from the Department of Zoology, Agricultural Experiment Station, Kansas State Agricultural College.

CAN EGGS SWALLOWED BY PIGS PRODUCE INFECTIVE LARVAE?

In the tropics and sub-tropics the ingestion of quantities of hookworm eggs by pigs is a daily occurrence. Countless numbers of these eggs are dropped in environments which doubtless enable them to hatch. But as the young, unsheathed hookworm larvae are known to be unable to penetrate the skin of their final host, the feces of the pig must furnish sufficient nutriment to enable the hookworm larvae to reach the infective stage, if the pig is a real factor in the spread of hookworm disease.

To test this point two cubic inches of human stool from Beggar No. 1 (light infestation), containing many hookworm eggs, were administered to the pigs. The feces passed during the following 24 hours were cultured in animal charcoal and placed in Petri dishes. In six to eighteen days afterward 72 active infective hookworm larvae were isolated from these cultures. This experiment was repeated by giving to the pigs 3 cubic inches of human stool. The feces collected after 16 hours were cultured in animal charcoal and placed in a large moist chamber. Examinations of this culture by washing from the 10th to the 17th day afterward yielded 289 infective hookworm larvae, apparently similar in every respect to hookworm larvae from human stools cultured directly in animal charcoal. These and many subsequent tests showed conclusively that hookworm eggs swallowed by pigs are able to produce larvae which develop to the infective stage in a culture medium of pig feces and animal charcoal.

As animal charcoal is known to inhibit putrefaction it appeared that the chances of young hookworm larvae reaching the sheathed stage would be less favorable in a medium lacking the charcoal. To settle this point, one and a half cubic inches of stool from Beggar No. 2 (heavy infestation) were administered to the pigs without any charcoal. The resulting feces were mixed with previously heated garden soil and placed in an open earthen pan. Examination by the aid of the isolation apparatus nine days later yielded 871 vigorous infective hookworm larvae. This demonstrated that hookworm eggs which had passed through the digestive tract of the pig produced infective larvae in the absence of animal charcoal.

WHAT PROPORTION OF HOOKWORM EGGS SWALLOWED BY PIGS REACH THE INFECTIVE STAGE?

Having determined that hookworm eggs swallowed by pigs produce infective larvae under approximately natural conditions, an attempt was made to obtain a comparison of the number of sheathed hookworm larvae developing from equal portions of a stool when one-half of it was cultured directly and the remainder given to a pig. For this series of tests Pig. No. 1 was chosen because of its voracious appetite. The essential data in these tests are shown in Table I.

TABLE I.

Comparison of culturing one half of stool directly with giving other half to pigs.

Cubic inches of stool.	Disposal of stool.	Feces recovered hours after feeding.	Culture medium.	Environment.	Days cultured.	Infective larvae obtained.
4..........	Half cultured		Soil	Shade	9	1,356
	Half to pig	21	Soil	Shade	8	3,003
3..........	Half cultured		Soil	Sun and shade	8	103
	Half to pig	24	Soil	Sun and shade	8	1,160
2..........	Half cultured		Soil	Sun and shade	11	18
	Half to pig	44	Soil	Sun and shade	10	897
3..........	Half cultured		Soil	Shade	8	957
	Half to pig	24	Soil	Shade	7	1,720
4..........	Half cultured		Soil	Shade	7	3,672
	Half to pig	25	Soil	Shade	6	2,520

For this series of feedings the stool of one individual, known as Beggar No. 2, was utilized on account of the large number of hookworm eggs per volume in it, and because of the relative freedom from eggs and larvae of other nematodes. The " half " stools are approximate, having been divided optically. The culture medium in each case was previously heated clay loam, and the culture dishes, eleven-inch tin pans (two inches deep), perforated for drainage. The "shade" environment was either deep shade out of doors or in a small yard house. In the "sun and shade" environment the cultures were placed out of doors, where they were exposed to approximately four hours daily of direct sun rays and to the tropical showers.

In the first test the feces were scratched into the surface of the soil and kept moist by sprinkling, except on two days when they were placed out in heavy showers. The second test was practically a duplication of nature, the human stool and pig feces being placed on top of the soil and set out of doors side by side. There were short heavy rains nearly every day and much sunshine. The relatively small number (103) of larvae, obtained from the human portion, was probably due in part to the rapid disappearance of the stool, no visible trace of which was left at the end of the third day. This was due to the combined action of fly larvae and the rain, and to the non-fibrous condition of the stool. If adverse climatic conditions prevailed, just as the hookworm eggs hatched, the exposed larvae in such a culture would probably perish before they could migrate to shelter. On the other hand, the fibrous pig feces, which were spread out by the rain, formed a disk-like covering over the surface of the culture, affording protection for the young larvae against the intense rays of the sun and the force of the heavy rains. The conditions of the third test were similar to those of the second one, the soft human stool disappearing during the third day, when most of the larvae are in a precarious condition.

That the rays of the sun and the beating rains are especially unfavorable to hookworm larvae in soft human stools is indicated by the results of the fourth and fifth tests. The conditions of these tests were similar to those of the second and third tests, except the meteorological ones of sun and rain. These cultures, kept in deep shade in the yard house and gently sprinkled, yielded many more larvae. The fly larvae appeared to be almost, though not quite, as numerous as they were out of doors. Just what effect the fly larvae have on the developing hookworm larvae is a problem in itself. They undoubtedly aid in making the stool porous, admitting oxygen more quickly, but they usually break the protective crust, and as they feed wholly on the stool, the amount of nutriment is certainly reduced. Since nutriment is an important factor in the development of hookworm larvae, a reduction in number in such fly-infested stools would be the natural course of events.

The majority of the fly larvae in the human stools were those of *Drosophila,* apparently attracted by the undigested pieces of banana and other fruits. A smaller number of flies were drawn to the pig feces which contained many oat hulls and plant fibers. These not only formed a protective crust, but probably kept the food particles more concentrated for the developing larvae. At any rate, these tests indicate that under the present conditions in Trinidad, hookworm eggs, swallowed by pigs, have fully as favorable prospects for producing infective hookworm larvae as do those that remain in the human stools deposited out of doors.

Uncontrolled factors and possible errors in technique of course may enter into these results. The optical division of the stools into "halves" is approximate rather than accurate, but differences in volume of four or five per cent. are readily detected. The distribution of the eggs in the stools may vary, but in each one of several different microscopic examinations of Beggar No. 2's stool, hookworm eggs were found in the first smear. A possible error in counting must of course always be considered, but the numbers of infective larvae obtained from the pig cultures were so large that a high percentage of the hookworm eggs swallowed by pigs are evidently able to produce sheathed larvae during the rainy season in the tropics.

FATE OF INFECTIVE HOOKWORM LARVAE SWALLOWED BY PIGS.

As pigs in feeding and drinking about the yards are likely to swallow human hookworm larvae in the infective stage it was deemed advisable to ascertain if such larvae might be disseminated by the pigs. This question arose early in the work, and on June 16, 2,841 active infective larvae were administered to the two pigs in milk, in a soup plate, care being exercised to have the larvae swallowed. The feces passed during the next 24 hours were separated and placed in a unit of the isolation apparatus. Many free-living nematodes, but not a hookworm larva could be obtained in this way. The feces were then broken up and prepared for sedimentation. A further search likewise proved futile, not a single hookworm larva being found.

Four days after the first feeding, a second test was made by giving to one of the pigs 2,700 infective hookworm larvae in milk, the latter being stirred while the pig was drinking it. A second volume of milk added was lapped up clean, leaving no doubt that most, if not all, of the larvae were swallowed. The feces voided by this pig during the next 22 hours were teased and placed in the isolation apparatus, but in this test, as in the first, not a single hookworm larva could be obtained. As a check on the isolation apparatus, 72 sheathed hookworm larvae were placed in some pig feces that were free from larvae. This excrement was placed in the isolation apparatus under conditions similar to those of the second test with the result that 26 sheathed hookworm larvae were recovered.

The third and final feeding of infective hookworm larvae was made on June 27, when 3,000 sheathed larvae were given to one of the pigs in milk as before. To guard against the possibility of the larvae escaping from the feces, the latter were made to fall on clean dry paper. Feces from this pig were collected during the following 25 hours, the last fecal deposit having been taken immediately after defecation. This excrement was teased and placed in the isolation apparatus, with the final result that no hookworm larvae could be found. The fate of such ingested larvae is, at present, unknown. It is probable that they either penetrate the wall of the digestive tract or die in the attempt, in which case they would be more or less completely digested. To determine the fate of those which might enter the wall of the alimentary canal further investigation is necessary.

STRONGYLOIDES STERCORALIS IN PIG EXCREMENT.

In addition to the abundance of hookworm eggs occurring in the human stools used in these experiments there were a few eggs of *Ascaris lumbricoides* and *Trichuris trichiura,* and an occasional larva of *Strongyloides stercoralis.* It was soon very apparent that the *Strongyloides* larvae could survive passage through the digestive tract of the pig, for in the cultures of pig feces these larvae in the free-living generation had multiplied to the extent of being numerous.

Among the results obtained by the Philippine Bureau of Science in 1913 from feeding human waste to pigs were indications that the pig may become a host of *Strongyloides stercoralis.* With numerous infective larvae of this species available from time to time in the present experiments it seemed desirable to attempt to collect more data on this problem. As stated elsewhere the feces of the two six-week-old pigs secured for the hookworm experiments were free from eggs and larvae on June 11. Many infective *Strongyloides* larvae were administered along with the infective hookworm larvae on June 16, 20 and 27. But on June 24 approximately 3,000 infective *Stron-*

gyloides larvae were given to one of the pigs. No visible effects of this dose were noted until three days later, when the pig refused to eat and vomited twice, remaining quiet the remainder of the day. By the next morning, however, the pig appeared normal again, and no further possible effects could be detected.

As time elapsed, the numbers of *Strongyloides* larvae in the pig feces cultures increased, indicating that the pigs were contributing some of the larvae. To test this point, the pigs were placed on a woven wire floor in the small feces recovery pen, where they were given food free from *Strongyloides* larvae. After allowing 28 hours or any ingested *Strongyloides* to pass from the pigs, the space beneath the wire floor was cleaned and covered by a clean paper. The ig feces were then collected for 18 hours and the major portion of hem cultured in previously heated soil, in the usual way, on August . In 8 days the culture was examined by the aid of the isolation pparatus. After centrifugation the centrifuge tube contained a solid ass of these larvae, approximately three centimeters deep. By diuting and stirring this mass of larvae, counting those in a single drop, nd multiplying by the number of drops, the total number obtained mounted to 68,400 *Strongyloides* larvae.

As a check one and a half cubic inches of Beggar No. 2's stool was ultured in previously heated soil and placed near the pig feces culure. Seven days later examination of this culture by aid of the solation apparatus yielded approximately 200 *Strongyloides* larvae. The contrast here is so great that there appears to be no other inerpretation than that these pigs were infested with mature *Stronyloides* of the parasitic generation. The 42 days which elapsed after he first feeding of infective larvae doubtless afforded ample time for evelopment to maturity in the pigs. While these tests do not prelude the possibility that the pigs became infested with a species of *trongyloides* other than *stercoralis,* the care and feed of the pigs was uch as to make it highly probable that it was the human species.

An interesting observation on the behavior of the free-living genration was made. On looking at the surface of one of the pig feces ultures some white wavy specks were noted. With the aid of a and lens it was seen that these specks consisted of groups of *trongyloides* larvae in the infective stage, the individuals being ound around one another, much like the strands of a thread, except hat at the extreme distal end of the group a portion of one larva xtended beyond the others. This and dozens of other masses were waving to and fro on the surface of the moist culture, certainly in favorable positions should the host come in contact with the surface of the culture.

HOOKWORMS IN PIGS.

Late in the season, an additional feeding of hookworm eggs was made in the usual way, one half of Beggar No. 2's stool being cultured directly and the other half administered to the pigs. The feces from this feeding were cultured and placed in the shade beside the human culture. Examinations of these cultures eight days later showed 1,200 infective larvae from the human culture and 6,203 from the pig culture. This latter number was so large—twice the number from any previous pig culture—as to lead to the suspicion that the pigs might be contributing hookworm eggs. Accordingly, a test was made in which the pigs were given carefully selected food for a day after which their feces were collected and cultured. In a week, the examination by the aid of the isolation apparatus yielded 4,294 infective hookworm larvae. These results tended to confirm the suspicion that the pigs had become infested with hookworms, and led to an immediate examination of the pigs' intestines.

In the intestine of the first pig slaughtered (Pig No. 2) five small blood clots were found in the ileum, and near them eight mature hookworms, five females and three males. The results from the examination of the second pig (No. 1) were similar, except that three mature hookworms were found, two females and one male. To ascertain whether hookworms were occurring in pigs kept under ordinary conditions, a three-month-old pig was purchased and slaughtered. From the posterior third of its small intestine 137 hookworms were taken, six pairs being in the act of copulation. Further examinations of pig intestines from various parts of the island showed that the infestations were not limited to young pigs, nor to particular geographical areas. Data on these points are still being assembled, and will be published in a later article of this series.

Studies on the morphology of the hookworms from several of these pigs convince the writers that these nematodes belong to the genus *Necator*, but differ from *Necator americanus* and other species of this genus to such an extent as to constitute a new species. A diagnosis of the new species is here given, but a full description of it together with data on its occurrence and distribution in Trinidad will be published as a later number of this series of papers.

THE GENUS NECATOR, STILES, 1903.

Hookworms belonging to this genus have a small mouth capsule, narrowed anteriorly (ventrally) by chitinous plates. On each side of the base of the dorsal cone is a lateral chitinous plate or lancet with a smooth edge (not serrated); ventral lancets present as in *Ancylostoma*. No ridges occurring on outside of ventral wall. Opening of the dorsal oesophageal gland on tip of a cone (dorsal tooth) projecting freely into the buccal capsule. Bursa closed. Dorsal ray of bursa divided to its base.

Necator suillus n. sp.

Specific diagnosis.—With the characters of the genus. Inner edges of ventral plates rounded. Dorsal tooth very short. Lateral lancets broadly wedge-shaped in profile. Ventral lancets rather slender; dagger-shaped in side view; point toward base of dorsal tooth. Oesophagus comparatively short and slender.

Male 5.25 to 8.75 mm. long by .09 to .246 mm. in thickness; average length, 6.9 mm. Buccal capsule 57 to 75 microns long (dorso-ventrally), average 65; by 58 to 77 microns wide, average 68. Bursa funnel shaped. Dorsal rays short and comparatively stout; terminal branches of dorsal rays long and slender; externodorsal ray short and stout; posterolateral, mediolateral and externolateral rays short and slender; lateroventral and ventroventral rays stout. Spicules .423 to .471 mm. long and barbed at extremity.

Female 7.3 to 12.5 mm. long by .295 to .382 mm. in breadth; average length, 9.5 mm. Buccal capsule 68 to 89 microns long, average 74; by 67 to 94 microns wide, average 75. Vulva near equator of body, but anterior to middle. Eggs 56 to 66 microns long by 35 to 40 microns wide, in early segmentation when deposited; average length, 63 microns; average width, 37 microns.

Among the morphological differences between *Necator americanus* and *N. suillus* are the following: The new species is somewhat smaller; the buccal capsule much smaller proportionately. As a rule, the dorsal turn in the neck is not so pronounced as in *N. americanus*. The inner edges of the ventral plates are more rounded in *N. suillus*, and less angular than those of *N. americanus*. In *N. suillus* the lateral lancets are broadly wedge-shaped in profile, while those of the larger *Necator* are cusp-shaped. The ventral lancet is slender in side view, pointing toward the base of the dorsal tooth in *N. suillus*, while this lancet in the larger species is broader and points approximately toward the tip of the dorsal tooth. The dorsal rays in the new species are shorter, while their terminal branches are actually longer than these structures in the larger *N. americanus.*

DISCUSSION.

The finding of mature male and female hookworms in pig No. 1 stimulates a re-examination of the data shown in Table I, and of the results from the preliminary feeding experiments. Among the questions which arise is that of the time at which these hookworms probably matured. As they are of the smaller species, *N. suillus,* they probably had entered the bodies of the pigs before June 11, when we secured them and found that their feces were free from hookworm eggs. The preliminary feeding experiments described above were begun at once, and the comparatively large numbers of infective hookworm larvae obtained from the pig cultures stood out in marked contrast to the small numbers obtained from similar chicken cultures (Ackert, 1922). The 871 vigorous infective hookworm larvae from preliminary pig feedings were obtained from feces passed four days after the pigs were secured. It will be seen that the one and a half cubic inches of Beggar No. 2's stool administered to the pigs in this case produced about as many infective hookworm larvae as developed in the average human cultures shown in Table I. This would indicate that the worms had not matured when this feeding was made, and it would also indicate that a high percentage of the human hookworm eggs swallowed by pigs are able to produce infective larvae during the wet season in Trinidad.

The tests for the data of Table I were begun June 19 when the pigs were about seven weeks old. If *N. suillus* matures as quickly as *N. americanus*, it is possible that the three hookworms found in pig No. 1 had matured at this time. On the other hand, they may not have matured until late in the season—the last of July—when 6,203 infective hookworm larvae were produced in a single pig culture, as compared with 1,200 from the corresponding human culture. However, as Calmette and Breton (1905) have shown that human hookworm eggs pass through the digestive tract of the dog unharmed by the stomach, intestinal, and pancreatic juices, there appears to be no reason for doubting the validity of the preliminary tests in the present experiments. These tests indicate that not only do human hookworm eggs pass uninjured through the alimentary canal of the domestic pig, but the majority of them produce infective larvae in a culture medium of pig feces and moist soil.

Anchylostoma in Swine.

After the preparation of this manuscript some references on *Anchylostoma* in swine came to our attention. O'Connor (1920) working in Funafuti, Ellice Islands, found thirty specimens of *Anchylostoma* in a pig. According to this author, the worms were very similar to *A. duodenale,* except for size, being smaller than this human hookworm. In July, 1921, Maplestone reported on the examination of 157 slaughtered pigs and on 25 fecal examinations of swine in North Queensland. No hookworms or eggs were found in any case. As only "the duodenum and first part of the small intestine, with its contents," were examined, any hookworms in the ileum and posterior portion of the jejunum of the slaughtered pigs would not have been found. In Trinidad, the present writers found *Necator suillus* more frequently in the ileum and jejunum than in the duodenum. Apparently, O'Connor found some *Anchylostoma* in the ileum and jejunum. More recently, Legg and Rheuben (1921) have reported the finding of hookworms in three semi-domesticated pigs from North Queensland, stating that the nematodes obtained had been identified as *Anchylostoma duodenale.*

SUMMARY.

1. Human hookworm eggs swallowed by domestic pigs produce infective larvae in five days during the rainy season in Trinidad.
2. The results of tests indicate that a high percentage of the hookworm eggs ingested by pigs are able to produce infective larvae, and that the free-range pig is an important factor in the dissemination of human hookworm eggs.
3. Infective hookworm larvae swallowed by pigs do not pass through unaltered in the excrement.
4. *Strongyloides stercoralis* larvae survive passage through the digestive tract of the pig and multiply in the feces. Circumstantial evidence shows that the pigs became infested with this nematode.
5. A new species of hookworm, *Necator suillus,* is of common occurrence in the domestic pigs in Trinidad, British West Indies.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. VI.

A Study of the Effect of Hookworm Control Measures on Soil Pollution and Infestation in a Sugar Estate.*

By WILLIAM W. CORT, Ph.D., and GEORGE C. PAYNE, M.D.

(Received for publication, December 13, 1921.)

Introduction.

In considering the aetiology of hookworm disease, the fact is soon apparent that the factors involved in the passage of the infestation from one individual to another are very complex. In order to ascertain accurately how the disease is spread in any given area it is necessary to consider (1) the extent and degree of the infestation of the people, (2) the prevalence of soil pollution, (3) the distribution of soil infestation and (4) the habits of the people with regard to possible effective contacts with the places of soil infestation. The investigation, the results of which will be given in this paper, is an epidemiologic study of a heavily infested area of a sugar estate. In this study an attempt was made to investigate all of the above-mentioned factors, both before and after the application of control measures, in order to determine the exact sources of the human infestation, and to learn the effect on human infestation, soil pollution and soil infestation of the control measures used.

DESCRIPTION OF THE AREA OF THE SUGAR ESTATE CHOSEN FOR STUDY.

The area chosen for this study (see map and Fig. 1) was a part of a sugar estate about one mile east of Prince's Town, Trinidad, British West Indies, and consisted of a set of thirteen barracks and their immediate surroundings. The barracks were on a hill and formed a definite unit set off from neighboring habitations. The barracks with their yards were bounded on the south by the main road, on the east by an estate road, on the north by a steeply sloping meadow and on the west by the edge of a sugar cane field, part of which was included in the study.

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* This paper is the sixth of the series on the hookworm investigations of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

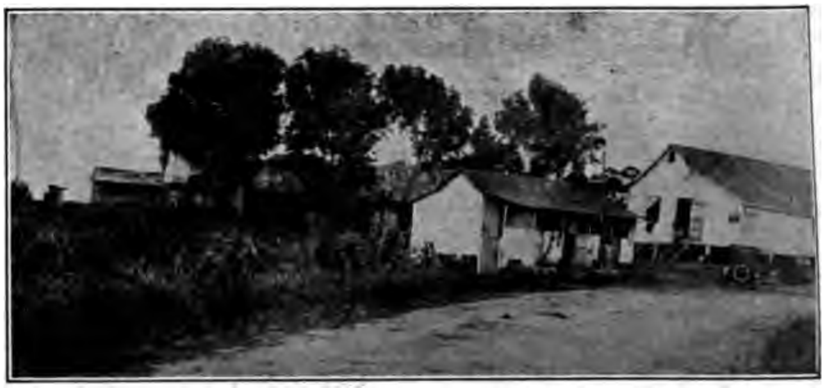

FIG. 1. View taken from the road, showing the relation of the barracks to the edge of the cane field.

The slopes in this area are quite steep. It is separated into two parts by a ridge running from north to south, just east of barracks 2, 11 and 13. The part east of this ridge drains to the east sharply, and that to the west drains from three directions into a gulley running between barracks 1 and 13 and continuing into the cane field. At the beginning of the work, the drainage around the houses was very poor, but it was later improved by the cleaning out of the old ditches and

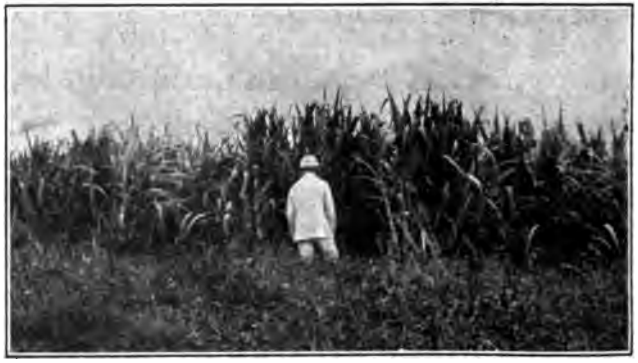

FIG. 2. View showing the size of the cane at the beginning of the work and the width of the rows.

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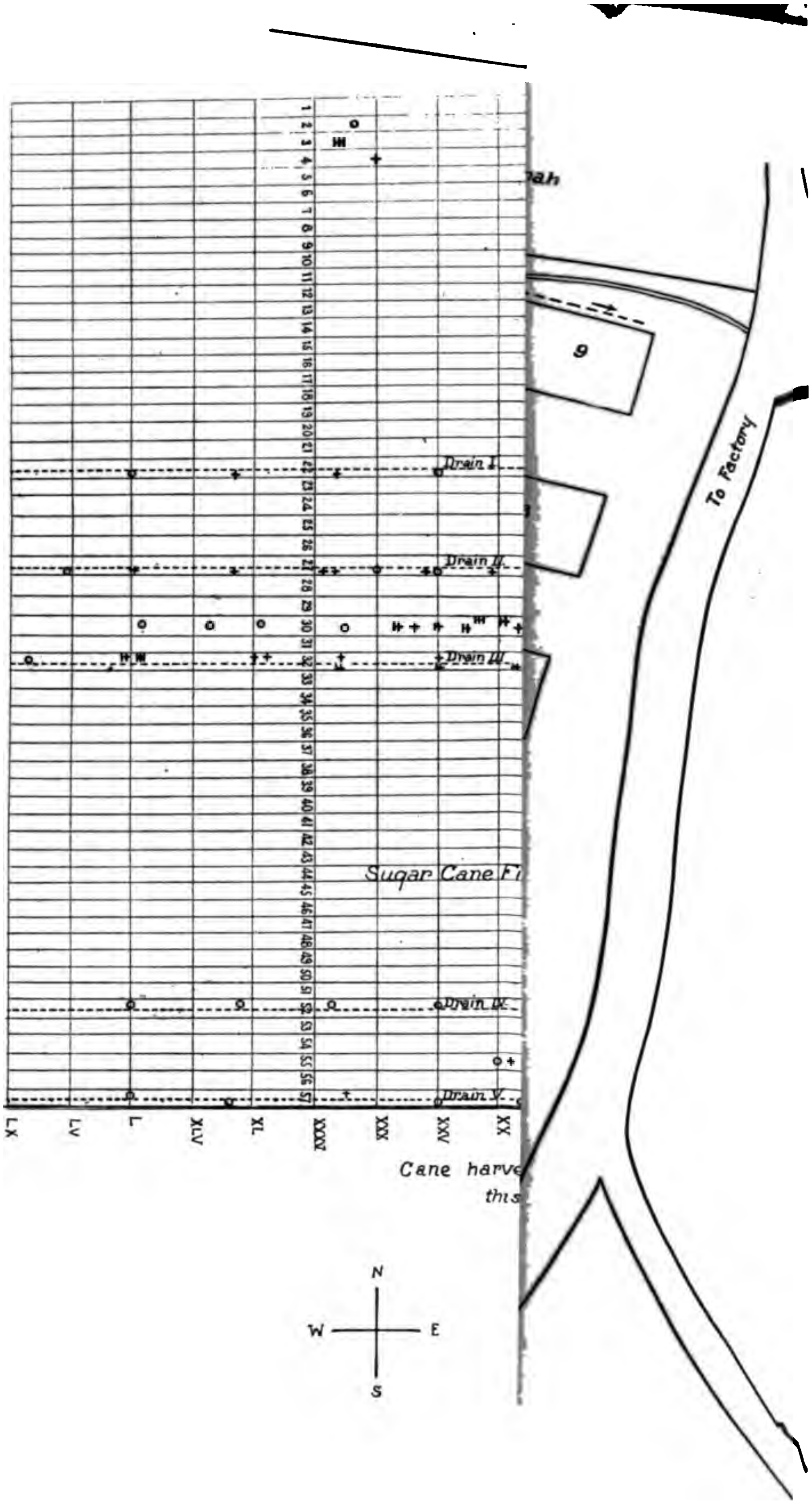
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the digging of new ones. The soil was a clay loam which adhered readily to the feet. After a rain the whole area, even in the paths, was very muddy.

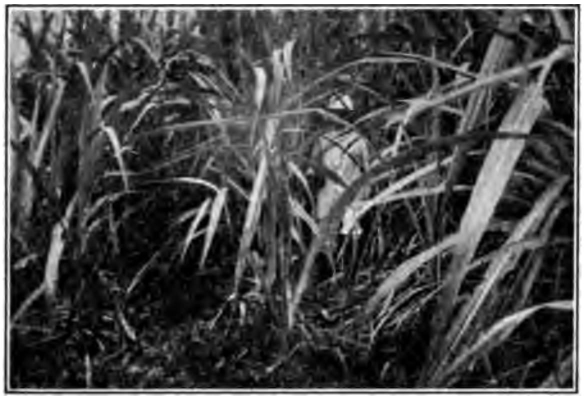


FIG. 3. View showing the kind of cover afforded by the cane.

There were a few trees around the barracks, and dense grass covered all the area. There was, however, no cover except that afforded by the cane field to the west. This field was not fenced and its wide rows were easily entered (see Figs. 2 and 3).

At the beginning of our investigation the buildings consisted of thirteen barracks, a large cow shed, a small shelter for cattle which was soon torn down, and two latrines of two units each. Later three more latrines were built, making a total of five latrines of fourteen units. The location of these buildings is shown on the map. The barracks were of two general types, as shown by Figs. 1 and 4. Those along the two roads were new and in good condition, but numbers 11,

EXPLANATION OF MAP OF AREA OF SUGAR ESTATE.

In this map the barracks are numbered from 1 to 13. In the cane field to the left on the map the parallel lines indicate the rows, which are numbered consecutively from north to south. The number of yards from the edge of the cane field is indicated by the Roman numerals along drain 5. The paths are shown by two parallel lines and the drains by dotted lines. The position of every soil sample taken from the area and the number of infective hookworm larvae present in the samples are shown by the following signs:

No larvae = 0 11 — 30 = ± 100 — = *
1 — 10 = + 31 — 100 = $\overset{-}{\underset{-}{+}}$

12 and 13 were in the last stages of dilapidation, as shown by the photograph of barrack 13 (see Fig. 4). All had wooden floors, but the open galleries were floored with packed clay.

Animals ranging over this area were almost entirely limited to

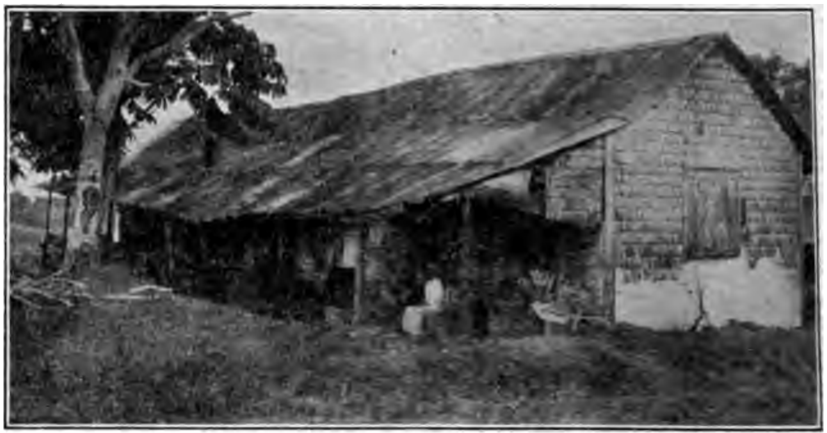


FIG. 4. Barrack 13.

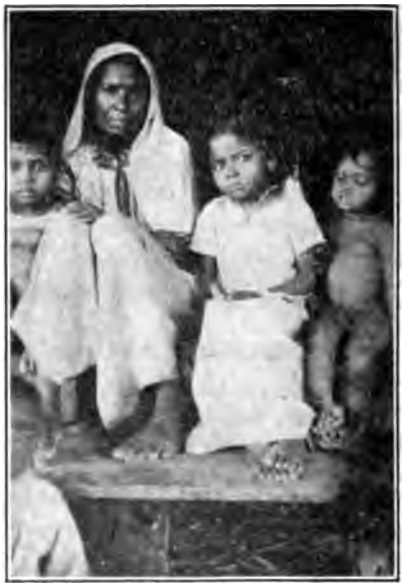


FIG. 5. A mother and three children from barrack 13.

cattle and goats, which were staked out to graze on the luxurious grass, but did not have free access to the cane.

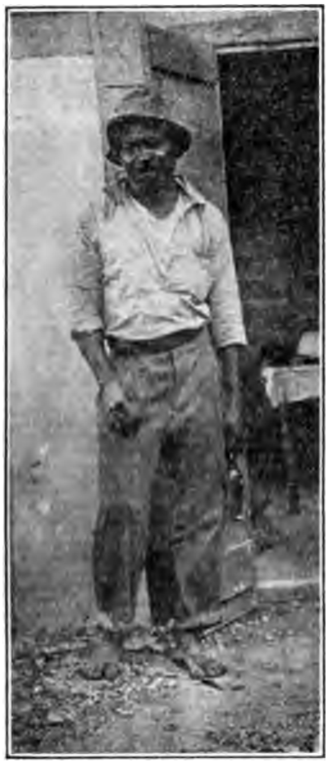


FIG. 6. Negro laborer of better type, showing bare feet covered with mud.

In all 146 people, the estate laborers and their families, lived in the thirteen barracks. Of these, 42 were negroes and 104 East Indians. There were 42 children under 12 years of age, and of the remaining 104 individuals 58 were males. Practically all the people, except the very young children, the aged and those incapacitated by illness, worked in the fields. This group of people (see Figs. 5, 6, 7 and 8) was characteristic of the type of estate laborers found in Trinidad.

INFESTATION OF THE PEOPLE.

To discover the individuals infested with hookworm disease in this area the technic of diagnosis commonly used in the hookworm campaigns of the International Health Board in the West Indies (Howard, 1919) was used. This method consists of the examination of two smears and the centrifugation of the negatives, followed by the examination of three smears of the sediment before a sample is finally declared to be negative. In order to make it possible to compare the mass infestation before and after treatment an attempt was made to roughly estimate the output of eggs. A record was kept of whether the particular specimen was positive on the first or the second smear or after centrifugation. If the specimen was positive on the first smear, a count of the number of eggs present in a smear 22 mm. by 16 mm. was made. In this way a rough comparative estimate was made of the intensity of the infestation.

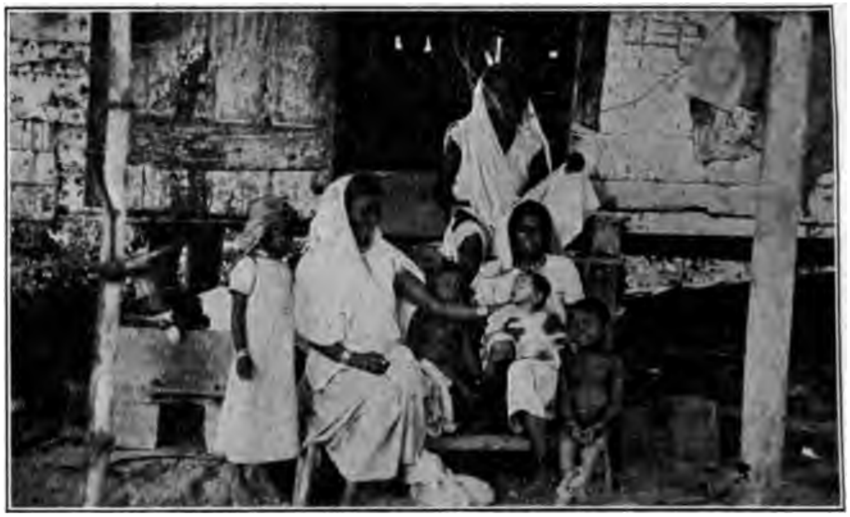


FIG. 7. Group of people from barrack 13. The little girl to the left was in a serious condition from hookworm disease complicated with malaria.

We realize of course that this method is much less accurate in estimating the reduction of infestation than the worm count method used by Darling and his associates (Darling, Barber and Hacker, 1920). Baermann (1917*a*) used the method of comparative haemoglobin estimates to test the result of his treatments in reducing infestation in the epidemiologic studies which he made in Sumatra. Since it was not possible to use either of these methods under the conditions of our investigation, we had to be content with a less accurate measure of the reduction of human infestation.

Out of the 146 people living in the area, 142 were examined for hookworm disease. Of these 117 were found to be positive and 25 were negative. Of the positives, 98 were diagnosed on the first smear with an average of 24 eggs per smear, 15 were diagnosed positive on the second smear, and 3 after centrifugation. A comparison of the negroes and East Indians showed no significant difference in the degree of infestation. Also a comparison of men and women showed nothing striking. Children under 5 years of age were found to be much less heavily infested than the rest of the population, for out of 18 children below this age only 7 were found to be positive.

FIG. 8. One of the old men from barrack 11.

The treatment of the people of this area started on July 22d. Each patient was given three treatments at intervals of a week before re-examination, which was made at least ten days after the last treatment. Each treatment consisted of a preliminary purge of magnesium sulphate administered at bed time, followed by a small dose of thymol at 6 A.M. the next day and a dose of oil of chenopodium, accompanied by a saline purge two hours later. Each dose of medicine was administered by a nurse, and the patients were kept under observation for several hours after treatment. The dosage used was on the basis of 30 grains of thymol or 30 minims of oil of chenopodium for a strong adult negro. Treatments were prescribed for each patient after a physical examination.

The record of the re-examinations, after treatment, shows a large number of negatives and a considerable reduction in the degree of infestation of those still found to be positive

Of the 117 individuals found positive in the preliminary examinations 110 were treated, 102 being given three treatments, 3 two and 5 only one. Of the 110 individuals treated 96 were re-examined after an interval of at least two weeks, to determine the effect of treatment. Of these 96 re-examinations 52 were negative. Of the positives 24 were found on the first slide with an average of 2.3 eggs per slide, 13 were found on the second slide and 7 after centrifugation. A comparison of these results with the preliminary examinations showed that a great reduction of mass infestation was brought about by the treatment.

SOIL POLLUTION.

It was the amount and general distribution of soil pollution over this area that first suggested that it would make a good place for an intensive study. When the first visit was made on June 22 there were two latrines (latrines No. 1 and 2), of the type shown in Fig. 9, for the use of the whole population. One of these latrines was full and was in an indescribably filthy condition. Feces was smeared all over the floor and in front and along the path leading from it. The other was not so bad, because it was apparently but little used. With only two latrines for a total population of 146, it is not surprising that soil pollution was found in every accessible place. For the purpose of discussion, the soil pollution conditions will be described first for the areas around the houses and yards, and second in the cane field, west of the barracks. At different times fresh stools were found in almost every situation around the barracks and in the yards. A special series of observations was made around barrack 13. Here on a mound, just two yards from the corner of the house, fresh stools were found day after day, sometimes as many as four on a day. Also in the area in front of this barrack stools could always be found.

In the vicinity of the latrines, especially around No. 1, there were always found stools. Soil pollution was common in the grass along the edge of the cane and in the grass in various places in the yards. Stools were even found in the paths and under the houses. The children were undoubtedly responsible for most of the pollution near the houses. The people said that it was impossible to control pollution by the children, which can readily be understood when we consider the conditions under which they live. The pollution in the yards was for the most part scattered and not concentrated into definite spots, which were visited day after day.

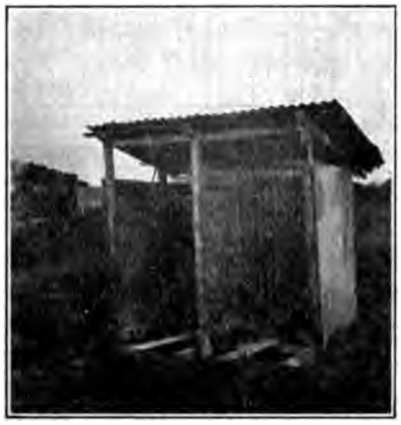


FIG. 9. Latrine number 2 at the time of the first visit to the area.

In the sugar-cane field, west of the barracks, conditions were studied more intensively, and pollution surveys were made during the whole course of our investigations in this area. The method of making a pollution survey in the cane was very simple. Charts were made indicating the position of the rows, the drains and the distances by yards from the edge near the barracks up to thirty-five yards into the cane. The persons making the survey then walked through this portion of each row and indicated on the chart the position of each stool found. The distance of the stools from the edge was measured by the number of paces. Fifteen such surveys were made of the northern half of the strip of cane bounding the area on the west (see map), of which seven were made before the construction of new latrines and the educational work had appreciably influenced the pollution.

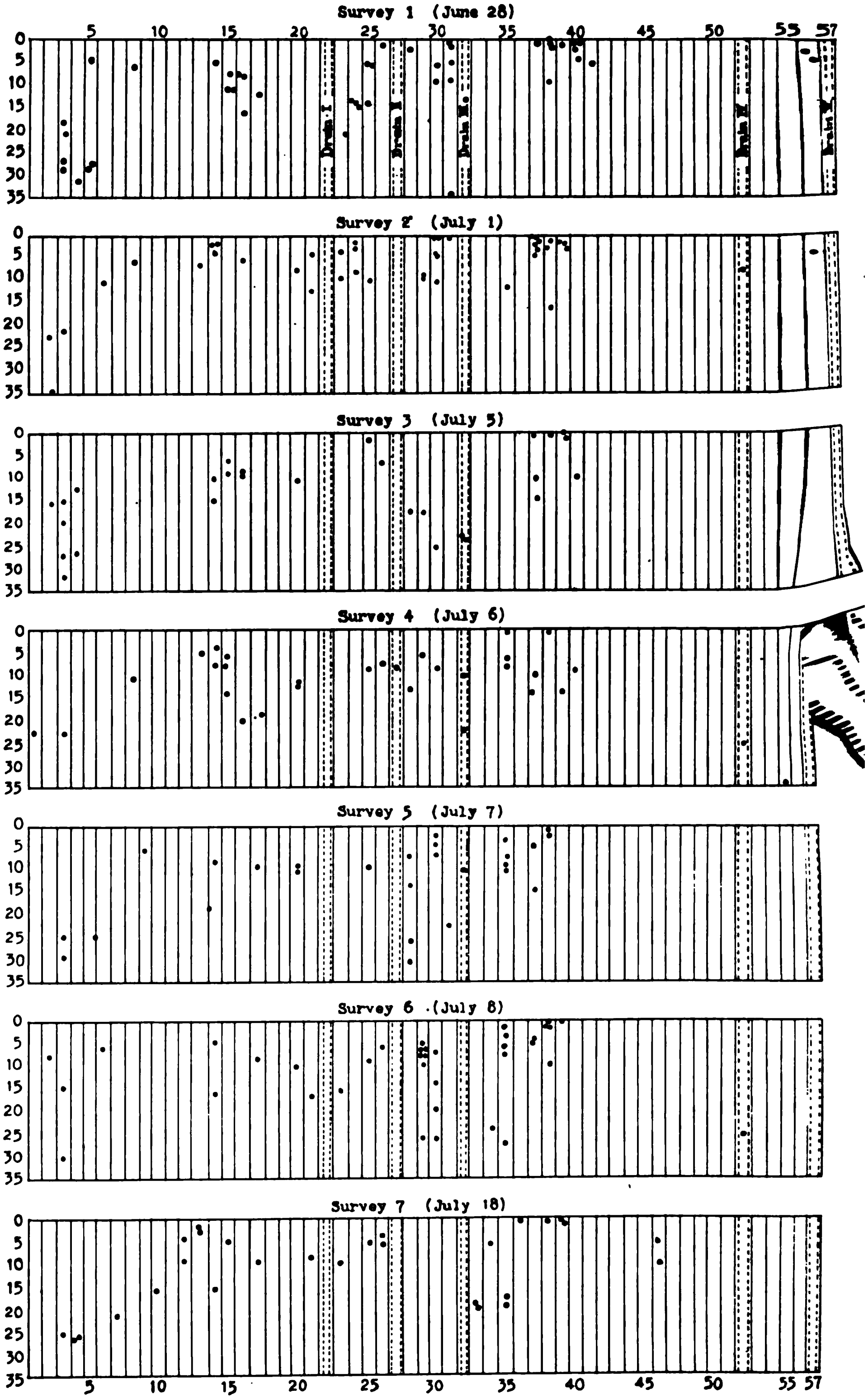
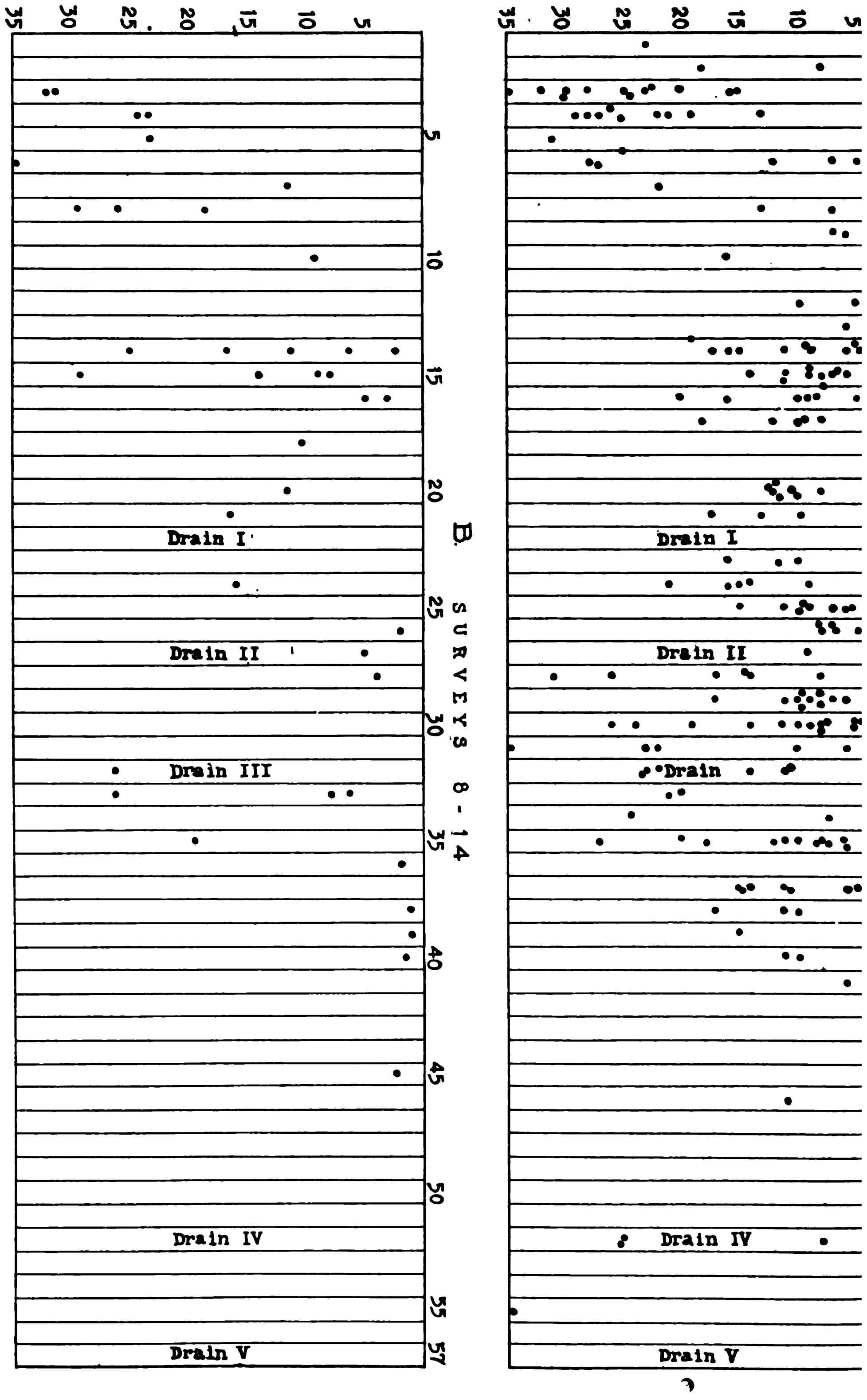

Survey 1 (June 28)
Survey 2 (July 1)
Survey 3 (July 5)
Survey 4 (July 6)
Survey 5 (July 7)
Survey 6 (July 8)
Survey 7 (July 18)
0
5
10
15
20
25
30
35
40
45
50
55
57
Drain I
Drain II
Drain III
Drain IV
Drain V

Chart 1 gives the results of these seven surveys. These surveys will be taken to represent the original conditions in the cane field. Chart 2 shows graphically the distribution of stools found in the seven original surveys. This diagram shows that while the pollution was considerably scattered there were definite centers of concentration, probably brought about by the easiness of access and proximity to the barracks. A number of points in our findings indicated that it was a common thing for a certain individual to visit the same place day after day, which would also tend to concentrate the pollution. This production of pollution centers in the cane was very important, since in this way were produced centers of soil infestation which were visited by the people of the area. It will be noticed from the chart that the great majority of the stools were deposited within fifteen yards of the edge of the cane. This holds for the pollution in rows 1 to 5, as well as elsewhere, since there was a large open space in this corner of the field, so that the edge of the cane was from five to twenty yards from the edge of the field. In the early surveys it will be noticed that numbers of stools were deposited from twenty to thirty-five yards from the edge of the cane. This was made possible by the fact that the cane was far from full grown at the time of these surveys, and it was easy to go down the rows (see Figs. 2 and 3). Later the growth of the cane made access to many of the rows practically impossible. This factor would tend to increase the concentration of pollution and therefore the danger of infestation. The position of the centers of pollution, as shown on Chart 2, can be readily explained. Rows 13, 14, 15 and 16 were fairly wide, giving easy access from barrack 13, and offered good shelter. The same condition with relation to other barracks held good in such rows as 20, 25, 26 and 35. The pollution in rows 29, 30 and 31 was concentrated in an open space protected by a fringe of cane at the edge. This place was very heavily polluted early in the work, but later the pollution shifted away from here, as will be described later. One

CHART 1. This chart shows the original seven pollution surveys made in the cane field area before the introduction of the educational campaign. The figures to the left indicate the number of yards from the edge of the field. The rows are shown by parallel lines, and the numbers of the rows are given along the top and bottom of the page. The position of the drains is shown by two parallel dotted lines, and the numbers are given in survey 1. The dots indicate the position of fresh human stools found on the morning of the surveys. All the surveys were made from 9 to 11 A. M.



B. SURVEYS 8 - 14

explanation for this was that while at the beginning of our study very little grass was present in this area, later it grew up to a heavy growth, which might have easily become objectionable to those visiting the place. The pollution center in rows 37 to 39 was an open space at the edge, where there was no cover. While both East Indians and negroes were known to visit the cane, it is to the former that the pollution of the cane was chiefly due. Besides the pollution shown on the diagram there was another center found in row 55, fifteen yards from the edge of the cane. A stool and evidence of previous pollution was found at this spot on June 22, but on no later date were other stools found, although the place was observed at every subsequent visit to the area.

A possible source of error in the pollution surveys was the difficulty in determining how old the traces of stool were. The plan of the survey was to count only recent stools in order that only a single day's pollution would be recorded in a single survey. Usually the stools disappeared with remarkable rapidity, but there was some delay in dry weather. Usually after twenty-four hours the stool would be leveled to the ground. In fact the loose soil of the cane field, the heavy rains and the intense activity of the insects made it possible for a very large amount of pollution to be absorbed into the soil in a short time.

Since it was possible to accurately measure soil pollution in the area of the cane just described, it was decided to continue the pollution surveys during and after an intensive hookworm campaign to test the results obtained.

Conditions which might influence soil pollution in the cane began to act as soon as we started our work in this area on June 22. It is possible that our early visits may have had an influence in keeping the people from visiting the cane field, but the pollution surveys, up to July 18th, show very little reduction in the daily deposit of stools. At the time of our second visit to this area on June 24, it was found that both the old latrines (Nos. 1 and 2) had been cleaned and moved,

CHART 2. This chart gives a summary of the surveys made in the cane field area. (*a*) Summary of surveys 1–7 (see Chart 1), which were made before the introduction of latrines and the control measures reduced the amount of soil pollution. (*b*) Summary of surveys 8–14 which were begun on July 22 after the introduction of latrines and educational work had reduced soil pollution and were continued at intervals until August 31 (see Chart 3). For further details see description of Chart 1.

and by about June 25 were put in fair shape for use. Soon after this, work was begun on the new latrines, and by July 9th four new units, latrine No. 5, were completed and in use. By July 15, the second four new units (latrine No. 4) were completed and soon were brought into use. By July 20 the last of the new latrines (latrine No. 3) was completed, and 14 units in five separate latrines were in use (see map). These latrines were in use up to the end of our work, and although they were at times allowed to become dirty, they represented a great improvement over the previous condition. That the bringing of these latrines into use did not at once reduce appreciably the pollution in the cane is shown by the surveys of July 8 and July 18, in which there was shown very little if any reduction.

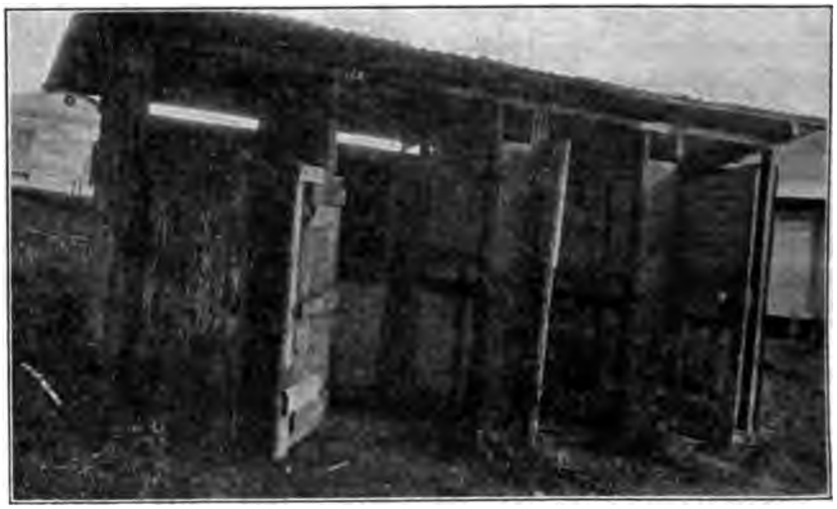


FIG. 10. Type of latrines built by the manager of the estate during the course of the work. Those of the seat type were reserved for the negroes, and of the squat type for the East Indians.

The type of new latrines built in this area is shown in Fig. 10. Special stalls were reserved for East Indians and negroes, with signs above the doors. The units reserved for the Indians had squatting platforms instead of seats. The pits under these latrines were about six feet deep and three feet wide. They were surrounded by banks of earth, outside of which were drains to divert the flow of surface water from the pits.

To supplement the introduction of the latrines educational work was instituted. On July 13 a trained East Indian was sent into the area. On this day and several days following he held demonstrations. In these demonstrations an attempt was made to acquaint the people with the effects of hookworm disease, its cause and methods of control. Especial emphasis was laid on the relation of soil pollution to the spread of the disease. To aid in the demonstration this worker was provided with an album of photographs and a series of colored charts, showing in an oriental allegory the main facts about hookworm disease. By arranging the demonstration in the afternoon, after the people had returned from the field, the great majority were reached. After this first series of demonstrations nothing more was done until Tuesday evening, July 19, when one of us (Payne) gave an illustrated lecture in the open air. This lecture was divided into two parts, both illustrated by the lantern. The first part gave a brief outline of the cause and method of control of hookworm disease, and the second showed by a series of cases from Trinidad the effects of the disease and the results of treatment. The people were informed of the lecture by the field assistant, and an audience of about 250 attended, including most of the people living in the area being investigated. One of the chief points made in this early educational work was that to control hookworm disease cooperation was necessary, that the estate manager was building the latrines, that the doctors would give the medicine, and that the part of the residents of the barracks was to use the latrines and not to pollute the soil. The point was constantly urged that treatments could not start until there was a reduction of soil pollution. The effect of this educational campaign is shown clearly in the striking reduction of soil pollution in the cane that was found in the survey of July 22, when only eight stools were found, as compared with the twenty-seven on July 18. Later surveys, as seen in Chart 3, continued to show this greatly reduced pollution, up to the time of closing the work. The latrines during this period came into constant use and became quite dirty. They were cleaned at times, but no regular system of cleaning was worked out. While this did not influence the present study it must be pointed out that unless latrines are kept reasonably clean, it is difficult to hold the ground gained by the educational work in the reduction of soil pollution. Treatment started July 22 and continued for several weeks. From this time until the end of our work there was at the barracks daily a nurse, who, in addition to administering treatment, instructed the people and answered questions. This nurse on Sunday, August 14, gave a series of microscopical demonstrations, showing adult hookworms, eggs and infective larvae. These demonstrations were accompanied by further explanations on the methods of transmission and control of hookworm disease. During all the work, there was a most hearty and interested cooperation by the manager of the estate. The educational campaign just described is the usual educational campaign of the Trinidad Ankylostomiasis Commission in an East Indian district.

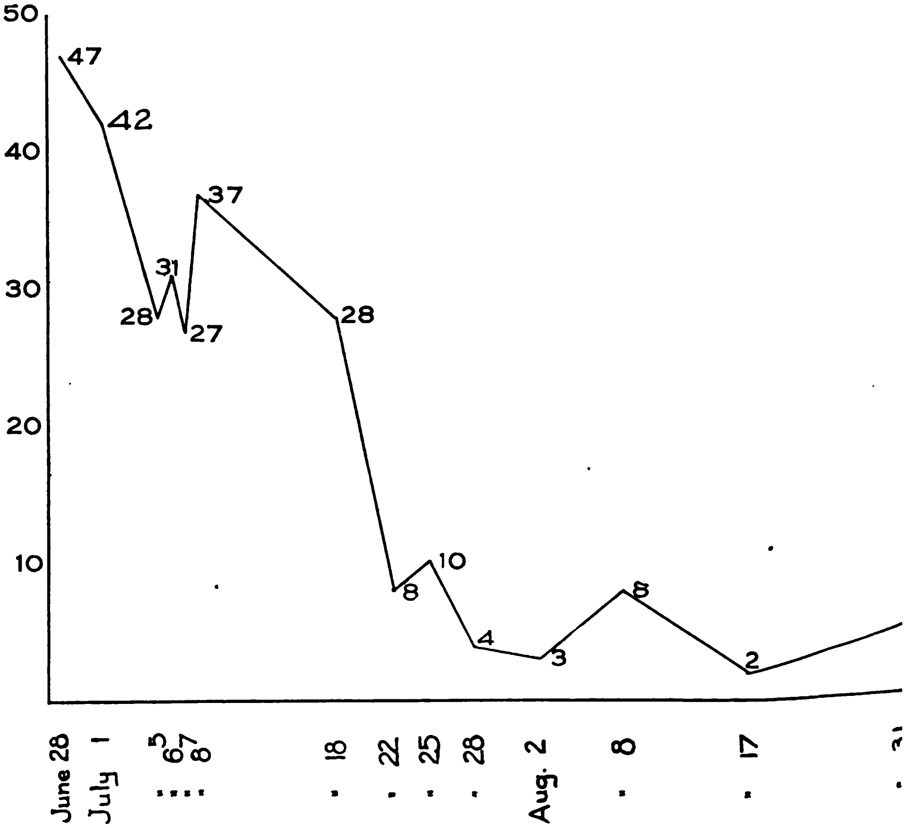


CHART 3. This chart is a curve to show the reduction of soil pollution in the cane field area, produced by the introduction of latrines and the educational work. The abscissa gives the length of time of the experiment, the units being one day each. The days on which the surveys were made are given. Along the ordinate is indicated the number of stools found.

No attempt was made to make the methods more intensive than those usually used. Therefore, the striking results in the reduction of soil pollution obtained illustrated (see Chart 3) what can be done by the usual methods, and is in reality merely a test of the effect of the routine educational campaign on soil pollution.

RESULTS OF EXAMINATION OF SOIL SAMPLES FOR HOOKWORM LARVAE.

The original purpose of the examination for hookworm larvae of soil samples from the sugar cane area was to determine the distribution of soil infestation, with the idea of locating the exact sources of the infestation of the people of the area. Incidentally data were obtained relating to the activities of infective hookworm larvae in the soil.

Attempts to discover the sources of human infestation usually consist of (1) a more or less accurate study of soil pollution, (2) an analysis of the habits of the people with regard to contacts with places that have been polluted, and (3) observations on the clinical side, especially in regard to ground itch. Although information from these sources is important, all such studies must be incomplete and often misleading, unless they include an investigation of the distribution of infective hookworm larvae in the soil. Up to the time of the researches of Baermann (1917*b*), the study of the distribution of infective hookworm larvae in the soil was practically impossible, because no apparatus had been devised by which they could be effectively isolated even from comparatively small quantities of soil. This difficulty has now been overcome (see Baermann 1917*b*, and Cort, Ackert et al., 1922), and it is now possible to locate the hookworm larvae even in comparatively large samples of soil. Baermann (1917*a*) in his extensive epidemiologic studies in Sumatra utilized the apparatus for the isolation of infective hookworm larvae from soil in the study of the sources of human infestation under a variety of different conditions. Most striking were his studies by this method of the sources of reinfestation in places where intensive control campaigns had been carried through, but where it had been impossible to eradicate the disease. His finding in this study of unsuspected and very important centers of soil infestation, in the wet spots inside of the houses (Baermann 1917*a*), shows how impossible it is to truly estimate the sources of human infestation without examining the soil for infective larvae.

The method, which we used to determine the location of hookworm larvae in the soil, was similar to that used by Baermann in his investigations. Samples of soil were brought in from the field in pint tin cans, set up in the isolation apparatus, and on the following morning the infective hookworm larvae obtained were counted. The technic of using the isolation apparatus was described in an earlier number of this series (Cort, Ackert et al., 1922). For recording the soil samples from the field special blanks were prepared as shown below.

SOIL SAMPLE RECORD.

No.

District *House No.* *Area*
Date *Time* *Rainfall for prior 48 hours*
Result of Examination: Hookworm Larvae *Free Nematodes*
Location

Soil Kind
Moisture Content: *Saturated* *Wet*
Moist *Slightly Moist* *Dry*
Shade
No Shade
Light Shade
Deep Shade
Vegetation

Possible Sources of Pollution

Danger to Inhabitants

Remarks:

These were given serial numbers to correspond with numbers placed with the soil in the tin containers and the necessary information filled in at the time the samples were taken. The locations from which the samples were taken were determined by the particular information desired. Since experiments by Augustine (1922*b*) and others of the Trinidad expedition had shown that as long as the soil is moist, the infective hookworm larvae were almost exclusively found at or very near the surface, only the surface layer was included in the sample. Usually the dirt for a single sample was obtained by scraping the surface of a small area or from several small spots over a somewhat larger area.

In all 233 soil samples were examined from the area under consideration. It will be impossible to include in this account all the data from the blanks which were filled out. A complete list of the samples and a summary of the results of the examinations is given in Tables 1 to 7.

Since the soil of this area was a clay loam, varying only in the percentage of clay present, the type of soil in all the samples was practically the same. Since the examinations were made during the wet season the soil was always at least moist and usually wet or saturated. The conditions of shade and vegetation, at the places where the samples were taken, seemed to be a factor in the presence of larvae. It was impossible to include these data for each sample, and they will be discussed for general groups of samples. Also the " possible sources of pollution " and " danger to inhabitants " need not be taken up for each individual sample. In practically every sample examined there were found free living nematodes varying in numbers from a few to several thousand. In addition there were found several different types of sheathed nematode larvae, which were thought to belong to Strongylidae of the cattle and goats on the area. To aid in the identification of the infective hookworm larvae, a few drops of 5 per cent. formalin would be mixed on the slide; this killed the free-living nematodes and made it much easier to study the preparations. A more detailed account of the technic of examining samples is given in an earlier number of this series (Cort, Ackert et al., 1922).

It will be noted from the summary (Table 1) that of the samples from the area 159 were taken from the strip of the cane field which was studied, and 74 were taken from places near the houses or in the yards. The map of the area shows graphically the location of the samples taken and the approximate results of the examinations. We will discuss first the samples taken outside the cane field and later the more intensive examinations of the strip of the cane field. As noted previously (Cort, Augustine et al., 1922) a large proportion of the infective hookworm larvae found were unsheathed.

The examinations outside the cane field developed into a hunt for larvae and the soil samples were taken with relation to known polluted places, places where pollution was suspected and places where human

TABLE 1.

Summary of samples from area.

No.	Location.	−	+	Hookworm Larvae Found.		
				Sheathed.	Unsheathed.	Total.
6	Drain 1	3	3	7	1	8
19	Drain 2	6	13	25	42	67
19	Drain 3	4	15	78	367	445
6	Drain 4	5	1	16	19	35
11	Drain 5	4	7	9	134	143
61	Drains 1–5	22	39	135	563	698
8	Rows 2, 3, 4, 6, 8	2	6	59	217	276
14	Rows 14, 15, 16	1	13	495	153	648
5	Rows 20, 23, 25, 26	0	5	93	111	204
31	Rows 29, 30, 31	7	24	172	196	368
21	Rows 35–40	11	10	238	180	418
19	Rows 55	8	11	601	1,052	1,653
98	Between Rows	29	69	1,658	1,909	3,567
21	Around Latrines	9	12	72	51	123
10	At ends of cane paths	4	6	6	14	20
43	Around houses	33	10	26	58	84
74	Total outside cane	46	28	104	123	227
61	Drains 1–5	22	39	135	563	698
98	Between Rows	29	69	1,658	1,909	3,567
159	Total from cane	51	108	1,793	2,472	4,265
74	Total outside cane	46	28	104	123	227
233	Total area C	97	136	1,897	2,595	4,492

contact with the soil was frequent. In this connection samples were also taken from around the latrines to test their importance as possible sources of infestation. The results of the examination of these samples are recorded in Tables 2, 3, 4 and 5, and the location from which they were taken is shown on the map of the area. The remarkable thing about these results was the small number of larvae isolated. The findings from the various types of situations will be discussed in some detail.

TABLE 2.

Wet spots under the galleries and near barracks.

No.	Date.	Location.	Larvae Found.		
			Sheathed.	Unsheathed.	Total.
208	July 25	B.2. W. side N. of steps under floor.	..	..	0
209	"	B.2. At sides of drain N. of house in front of second door from E...... .	..	..	0
210	"	B.3. S.W. cor. house around water barrel..........................	..	..	0
211	"	B.3. ditto.......................	..	..	0
212	"	B.4. Next to S.E. cor. of house... .	..	..	0
213	"	B.5. At S.E. cor. of house..........	..	..	0
214	"	B.6. Just out from S.E. cor. of house	..	..	0
215	"	B.7. Around boards directly W. of house..........................	..	..	0
216	"	B.8. Directly W. of house..........	..	.	0
217	"	B.9. Around bridge directly W. of house..........................	..	..	0
218	"	B.10. W. of middle door in drain around boards..................	..	..	0
118	July 8	B.11. S.E. cor. gallery, at inner end of bridge of iron pipes..............	..	..	0
119	"	B.11. S. edge of gallery, at inner end of pipes across drain..............	..	..	0
120	"	B.11. 2 yards E. of N.W. corner of gallery inside gallery.............	..	1	1
121	"	B.11. 2 yards W. of S.W. corner of gallery in path..................	..	..	0
122	"	B.11. 4 yards S.W. from 121 along path...........................	..	..	0
123	"	B.11. 5 yards S.W. 122, where path from house 11 joins path from 12...	..	..	0
124	"	Just at edge of gallery of cowshed where path crosses drain..........	..	..	0
125	"	B.12. S.E. cor. of gallery, just outside, across drain.................	..	..	0
126	"	B.12. At E. end of gallery next to house........................	1	..	1
127	"	B.12. Along outer edge of gallery next to drain.........................	..	..	0
128	"	B.12. At front of gallery in front of middle door, at inner end of bridge.	..	..	0
129	"	B.12. In gallery E. of 1st steps toward W. against house.................	..	..	0
130	"	B.12. At inner end of ditch just E. of bridge across drain at W. end......	..	..	0
131	"	B.13. Just W. of S.W. corner past house..........................	..	..	0

No.	Date.	Location.	Larvae Found.		
			Sheathed.	Unsheathed.	Total.
132	July 8	B.13. 3 yds. W. of house, where path from house crosses drain..........	..	..	0
133	"	B.13. At edge of gallery just W. of W. bridge.........................	..	..	0
134	"	B.13. At edge of gallery, opposite two W. water barrels, inner end of bridge	..	..	0
135	"	B.13. At inner edge of drain along gallery, W. of water barrels........	..	..	0

Stimulated by the findings of Baermann (1917*a*) in regard to soil infestation within and near houses a series of samples was taken near the barracks. Since all the barracks on our area had wooden floors, there were no conditions exactly comparable to the wet spots which Baermann found inside the "pondoks" in Sumatra. Similar to these places, however, were wet spots under the galleries, or just outside the shelter of the houses, often near water barrels where the people washed themselves or their utensils. Most of these places were shaded for all or part of the day, were constantly wet and often visited by the people. They were also alike in being without grass and in the soil being very solidly packed. No visible signs of pollution were found at any of these spots. Soil samples, totaling twenty-nine in number, were taken from all the places of this type in the area. The results of the examination of these samples are given in Table 2. It will be seen that there were only two positives, with one larva each. An analysis of Baermann's findings in regard to soil infestation of the wet spots indicates very strongly that situations of the type just described would under conditions in Sumatra have had a heavy soil infestation. In trying to explain the lack of soil infestation of these wet spots in our area several possibilities suggest themselves. In considering environmental conditions it would appear that there was plenty of moisture in these situations, and that many of them were continually shaded. The only unfavorable factors which suggest themselves are the type of soil which was a clay loam, rather solidly packed, and the lack of any vegetation on the soil. Since there was no evidence of pollution found at these spots it is of course possible that hookworm eggs and larvae were not brought to them in sufficient quantities to produce soil infestation. In one respect conditions differed from those found by Baermann in Sumatra, in that while the people there were accustomed to wash themselves after defecation, at the wet spots within or near the houses, in Trinidad it was the custom of the East Indians to carry a bottle of water to the place of defecation for this purpose. However, in view of the widespread soil pollution in this area, even close to the houses, and of the fact that the people washed their feet at these places, it is hard to believe that fecal material and larvae would not be carried there.

TABLE 3.

Polluted places near barrack 13.

No.	Date.	Location.	Larvae found.		
			Sheathed.	Unsheathed.	Total.
147	July 11	B.13. Mound W. of gallery, 2 yds. W. of gallery support	..	..	0
148	"	B.13. 2 yds. W. of 147, on mound	..	4	4
149	"	B.13. 1 yd. S. of 148, on mound	..	5	5
150	"	B.13. 1 yd. S.W. of 149	..	14	14
151	"	B.13. 3 yds. S. of middle of barracks, in path	..	..	0
152	"	B.13. 5 yds. S. on path from 151	2	2	4
153	"	B.13. In polluted area in front of barracks	3	1	4
219	July 25	B.13. In polluted area in front of barracks	..	..	0
220	"	B.13. In polluted area in front of barracks	..	..	0
221	"	B.13. In polluted area in front of barracks	20	27	47
222	"	B.13. In polluted area in front of barracks	..	3	3
223	"	B.13. In polluted area in front of barracks	..	..	0
224	"	B.13. In polluted area in front of barracks	..	1	1
225	"	B.13. In polluted area in front of barracks	..	..	0

The results of the examinations of a series of samples from a somewhat different type of location are shown in Table 3. These samples were taken near barrack 13, at places where there was a considerable amount of soil pollution. Samples 147 to 150 were taken on a mound, just west of barrack 13. This mound was a very heavily polluted spot, evidently a favorite place with the children. It received morning shade and was covered with sparse grass and debris from the house. With the amount of pollution here the finding of three out of four positive samples is not surprising. The rest of the samples in Table 3 were taken from an area in front of barrack 13 (see map). This area was planted to tannia, grown up thickly with weeds, and partly shaded by a large cedar tree. It was constantly polluted, especially by the children. The findings from this place show a considerable amount of scattered soil infestation, but certainly not commensurate with the amount of pollution. In fact in both of the areas from which the samples in Table 3 were taken, we were very much surprised at the small numbers of larvae found, as compared with the grossness of the pollution. It is possible that here too the conditions were not the best for the development of the larvae, although their small numbers might be explained by the fact that most of the pollution near the house was by children, who were not as heavily infested as the adults. The lack of uniform distribution of the larvae over the comparatively small areas near barrack 13 indicates that they do not migrate widely in the soil.

Table 4 gives the results of the examination of samples taken around the latrines. Samples 1 to 9 were taken with regard to the possibility of one of the original latrines being a source of infestation. The condition of this latrine (latrine No. 1) at the time of this examination has been described above. Since the whole inside was smeared with feces, and the whole area in front was polluted both with whole stools and feces tracked from inside, it can be seen that there was a very great concentration of soil pollution. In considering samples 1 and 2, taken directly in front of this latrine, it is surprising that more larvae were not found. In fact, the place where sample 1 was taken was almost constantly smeared with feces, and the fact that only two larvae were found indicates that the situation was unfavorable for development. The soil here was almost pure clay, closely packed and without grass. It was shaded in the morning, but exposed to the direct rays of the afternoon sun. Sample 2 was taken from the same situation, only one foot from 1, but differed in being taken around the roots of a clump of grass. A comparison of the findings from these two samples shows that in clay soil, especially

TABLE 4.

Near the latrines.

No.	Date.	Location.		Larvae found.		
				Sheathed.	Unsheathed.	Total.
1	June 22	Latrine I.*	1 ft. in front of door, ground bare	2	..	2
2	"	" I.	1 ft. south of 1, in grass.	19	32	51
3	"	" I.	Along path 1 yd. from 2	14	3	17
4	"	" I.	" " 3 yds. " 2	2	1	3
5	"	" I.	" " 7 " " 2	3	1	4
6	"	" I.	" " 10 " " 2	8	3	11
7	"	" I.	" " 15 " " 2	2	4	6
8	"	" I.	Directly back of middle.	7	1	8
9	"	" I.	1 ft. directly back of 8	2	4	6
309	Aug. 2	" I.	Along front	..	..	0
310	"	" I.	In path to south	..	..	0
311	"	" I.	Along path to south from 1 yd. to 6 yds.	..	..	0
302	"	" II.	Along front	..	..	0
303	"	" II.	Along path to west of 302	2	..	2
300	"	" III.	All along front	9	1	10
301	"	" III.	Along path to north	..	..	0
304	"	" IV.	Along front	..	..	0
305	"	" IV.	On path to north	2	1	3
306	"	" IV.	Along path to south	..	..	0
307	"	" V.	Along front	..	..	0
308	"	" V.	Along path to south	..	..	0

When packed hard, the presence of grass which gives protection and loosens the soil will make the situation favorable for the development of hookworm larvae. Samples 3 to 7 are of interest, since they show the possibility of the spread of soil infestation along a path. The path was covered with a dense grass and so conditions were favorable for development of larvae. It seems certain that this latrine under the conditions which existed prior to June 22 was a source of infestation with hookworms to those visiting it.

Samples 300 to 311 were taken at the most favorable places for pollution and human contact in connection with the new latrines which were installed in this area, after they had had time to become dirty. There was considerable fouling of the places from which these samples were taken before July 22 when the first treatments were instituted, so that there was opportunity for considerable soil infestation, if the conditions had been favorable. The only shade which the situations had was from the latrines, which was only effective for part of the day. Samples 300, 304, 306, 307 and 310 were taken from situations where there was no grass, while the others were protected by grass of various densities. Of the three positives only one (300) was unprotected by grass. This indicates that the larvae can occasionally develop in situations where there is very little protection.

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* Samples 1 to 9 were taken from original location, marked old pit on map.

In this whole series from around the latrines the small numbers of larvae found again indicate how difficult it is for soil infestation to develop, even with gross pollution by heavily infested individuals, when the soil is a hard-packed clay or clay loam. These findings in regard to latrines suggest that as a precaution against soil infestation the area immediately around a latrine should, if possible, be built up with a solid clay or cement platform and kept clear of grass.

TABLE 5.

At ends of paths into the cane.

No.	Date.	Location.	Larvae found.		
			Sheathed.	Unsheathed.	Total.
136	July 11	54th row. At edge of bank where path enters cane...........	..	..	0
137	"	" " 12 ft. directly east along path from 136.........	..	..	0
138	"	39th " At edge of bank on path into cane.............	3	..	3
139	"	" " 1 yd. up path from 138..	..	..	0
140	"	35th " At bottom of bank on path into cane.............	1	1	2
141	"	" " On path at top of bank...	..	..	0
143	"	33d " On path two yds. east of edge of cane...........	..	2	2
144	"	2d " On path at edge of bank at edge of cane...........	1	7	8
145	"	1½ yds. south of 144 along path.....	..	2	2
146	"	3 yds. south of 144 along path.......	1	2	3

Table 5 gives the results of examinations of samples taken at or near the ends of the paths leading into the cane field. Since we had no evidence of pollution in these paths it is probable that the results of the examinations illustrate the possibility of establishing soil infestation by the transportation of hookworm eggs and larvae on human feet. All these paths were covered with a dense growth of grass, but were otherwise unshaded. The possibility of transportation of hookworm larvae on the feet is brought out by a series of examinations of the mud from the feet of individuals visiting the cane field, which gave hookworm larvae in considerable numbers (see Augustine 1922*a*, p. 169).

To make an intensive study of the distribution of hookworm larvae in the cane field to the west of the barracks, a series of 159 soil samples was examined from the heavily polluted area extending for over a hundred yards along the west side of the barracks and for thirty-five yards into the field (see map). A detailed study of the soil pollution in this area was recorded earlier in this paper and should be considered in connection with the results of the examination of the soil samples. The samples were taken, except in the drains, in direct relation to the centers of pollution, and a comparison of Chart 2*a* with the map of the edge of the cane gives graphically an idea of the relation between soil pollution and soil infestation in this area. Soil samples were taken from three general types of situations, viz., (1) between definite rows of cane, (2) in open spaces at the edge or a short distance back into the cane, and (3) from the bottoms of the drains. The summary of the results of these examinations (Table 1) shows a very heavy soil infestation, for out of the 159 samples examined only 51 were negative for infective hookworm larvae, and 108 were positive. From these 108 positives, 4,265 infective hookworm larvae were isolated, of which 1,793 were sheathed and 2,472 were without their protective sheaths. The significance of finding such a large proportion of the infective larvae without their protective sheath has been discussed in another connection (Cort, Augustine et al., 1922). From this intensive study of soil infestation in this strip of cane field data were obtained on the relation between soil pollution and soil infestation and on the activities of infective hookworm larvae in the soil. We will discuss in some detail the results of the findings. For convenience the findings from between the rows will be discussed separately from those in the drains.

All the samples from between the rows were taken with relation to definite centers of soil pollution. The results of the examinations

TABLE 6.

From between rows of cane field strip.

Sample No.	Date taken.	Location.	Hookworm larvae found.		
			No. sheathed.	No. unsheathed.	Total.
191	July 18	R. 2; 32	..	..	0
82	“ 4	R. 3; 16	..	..	0
83	“ 4	R. 3; 21	31	2	33
190	“ 18	R. 3; 33	16	66	82
84	“ 4	R. 4; 30	..	4	4
85	“ 4	R. 4; 35	..	8	8
192	“ 18	R. 6; 12	12	136	148
193	“ 18	R. 8; 10	..	1	1
75	“ 1	R. 14; 3	1	..	1
88	“ 4	R. 14; 3	..	1	1
154	“ 13	R. 14; 4	1	36	37
76	“ 1	R. 14; 6	..	..	0
155	“ 13	R. 14; 7	2	9	11
77	“ 1	R. 14; 9	..	1	1
89	“ 4	R. 14; 11	407	..	407
78	“ 1	R. 14; 12	..	1	1
90	“ 4	R. 15; 5	2	4	6
156	“ 13	R. 15; 6	72	21	93
91	“ 4	R. 15; 7	2	6	8
157	“ 13	R. 15; 9	7	68	75
194	“ 18	R. 15; 13	..	4	4
195	“ 18	R. 16; 7	1	2	3
196	“ 18	R. 20; 10	18	10	28
197	“ 18	R. 23; 7	8	13	21
199	“ 18	R. 25; 5	2	1	3
198	July 18	R. 25; 10	..	35	35
200	“ 18	R. 26; 7	65	52	117
201	“ 18	R. 28; 3	1	..	1
202	“ 18	R. 28; 5	3	14	17
50	June 29	R. 29; 8	..	..	0
68	July 1	R. 29; 8	1	..	1
203	“ 18	R. 29; 10	35	5	40
158	“ 13	R. 29; 8	2	14	16
16	June 24	R. 30; 1	2	10	12
159	July 13	R. 30; 1	..	2	2
17	June 24	R. 30; 2	5	31	36
18	“ 24	R. 30; 3	..	2	2
19	“ 24	R. 30; 5	8	21	29
160	July 13	R. 30; 6	..	..	0
161	“ 13	R. 30: 7	..	..	0
20	June 24	R. 30; 7	2	24	26
162	July 13	R. 30; 10	..	8	8
21	June 24	R. 30; 10	7	13	20

TABLE 6—*Continued.*

Date taken.	Location.	Hookworm larvae found.		
		No. sheathed.	No. unsheathed.	Total.
" 24	R. 30; 13	..	7	7
" 27	R. 30; 16	15	2	17
July 13	R. 30; 18	..	1	1
June 24	R. 30; 19	6	13	19
July 13	R. 30; 22	29	4	33
June 27	R. 30; 22	16	7	23
" 27	R. 30; 25	18	7	25
July 13	R. 30; 27	..	1	1
June 27	R. 30; 28	10	5	15
June 29	R. 30; 33	..	..	0
" 29	R. 30; 38	..	..	0
" 29	R. 30; 43	..	..	0
" 29	R. 30; 48	..	..	0
July 13	R. 31; 5	5	..	5
June 29	R. 31; 10	7	5	12
July 13	R. 35; 4	20	..	20
" 13	R. 35; 9	2	15	17
" 13	R. 37; 1	2	..	2
" 6	R. 37; 2	13	..	13
" 13	R. 37; 3	..	4	4
" 1	R. 37; 3	..	..	0
" 6	R. 37; 4	36	6	42
" 1	R. 37; 6	..	..	0
" 1	R. 37; 9	..	..	0
" 18	R. 37; 12	161	150	311
" 13	R. 38; 1	..	..	0
" 1	R. 38; 1	..	..	0
July 13	R. 38; 3	..	..	0
" 6	R. 38; 4	..	..	0
" 18	R. 38; 8	..	1	1
" 13	R. 39; 1	..	..	0
" 6	R. 39; 2	3	1	4
" 13	R. 39; 3	..	..	0
" 6	R. 39; 4	1	3	4
" 18	R. 40; 5	..	..	0
" 18	R. 40; 9	..	..	0
June 29	R. 55; 1	..	..	0
June 29	R. 55; 3	..	..	0
" 29	R. 55; 7	..	..	0
" 29	R. 55; 10	..	..	0
July 13	R. 55; 11	..	..	0
" 13	R. 55; 13	..	..	0
June 22	R. 55; 14½	17	126	143

TABLE 6—*Continued.*

Sample No.	Date taken.	Location.	Hookworm larvae found.		
			No. sheathed.	No. unsheathed.	Total.
65	July 1	R. 55; 14½	208*	209*	417
10	June 22	R. 55; 15	312*	625*	937
64	July 1	R. 55; 15	18	35	53
186	" 13	R. 55; 15	. .	9	9
12	June 22	R. 55; 15[1]	20	17	37
67	July 1	R. 55; 15[2]	14	10	24
13	June 22	R. 55; 16	7	10	17
66	July 1	R. 55; 16	. .	1	1
14	June 22	R. 55; 17	5	7	12
62	" 29	R. 55; 17	. .	. .	0
15	" 22	R. 55; 19	. .	3	3
63	" 29	R. 55; 20	. .	. .	0

* Approximate count and estimate.

[1] 1½ ft. north from 10.

[2] 1 yd. south from 10.

of these samples are given in Table 6. A study of this table in connection with the map of this area will give a pretty clear idea of the distribution of soil infestation in the cane. Most striking perhaps is the very large number of larvae isolated from this area and their concentration at places of intense pollution.

That even in the cane area soil infestation is definitely limited by the effect of the environment on the development of the larvae is shown by the comparison of soil pollution with soil infestation. A very striking example of this point is the condition at the open space, at the edge of the cane, at rows 37, 38 and 39. This area extended about four yards back from the edge and during the early examination was practically without vegetation. The ground at this place was packed hard and there was a high proportion of clay in the soil. This place, with an area of about twenty-four square yards, as can be seen from Chart 2*a*, was the most heavily polluted spot in the cane field, for in the seven original pollution surveys a total of thirty-two stools were recorded. Yet the examination of Table 6 shows that, as compared with other pollution centers, it had a very light soil infestation. A total of thirteen soil samples, on three different days, July 1, 6 and 13, were taken from this place, viz., samples 169, 102, 181, 79, 103, 170, 104, 182, 105, 171, 106, 183, 107. Seven of these were negative and six positive. Only one of the positives (No. 103) had more than a few larvae in it, which might be explained by the fact that this sample was taken from the bottom of a shallow hole which was somewhat protected and received drainage from around. The only explanation we are able to suggest for this very small soil infestation, from a very heavy soil pollution by individuals infested with hookworms, is the hard packed character of the soil and the open unprotected character of this place, where there was no shade and practically no grass or weeds. A comparison of the soil infestation of this place with that of other less heavily polluted spots in the cane, which were protected, shows clearly the importance of shade and vegetation in producing conditions favorable for the development of hookworm larvae. Such a finding illustrates how impossible it is to judge soil infestation or the sources of human infestation by the distribution of soil pollution alone, since not pollution, but pollution plus proper conditions for development, make soil infestation possible.

The findings from the soil samples in the cane field give some significant data on the question of the migrations of the infective hookworm larvae from the place of their development. The soil of the cane is a loose clay loam, containing a considerable proportion of humus, and so light that it would appear to give a favorable medium for the migration of any living organism found in it. The ordinary conception is that infective hookworm larvae are able to migrate actively to an appreciable distance from their place of development and infest the soil over a considerable area. If this were true we would have expected to find a fairly even distribution of the larvae over large portions of the area examined. Instead of this the results show a very definite localization of soil infestation at the places of soil pollution. In a number of cases of two samples taken close together one gave a large number of larvae and the other was negative or contained but few larvae. Several examples from Table 6 will serve to illustrate this point. In row 14 samples 77, 89 and 78 were taken from the center of the row within a radius of three yards. From No. 89, 407 infective hookworm larvae were isolated, while the other two contained only one each. Further examples of the same thing can be obtained from the records of the examination of soil samples from rows 29, 30 and 31. In these three rows there was an area of heavy soil pollution especially in an open space from about two yards to ten yards into the cane. Yet within this area of general soil pollution and soil infestation certain samples taken were found to be negative or almost so. This is strikingly illustrated by samples 50, 68 and 18. Perhaps the best example from this area of failure to migrate is shown in the results of the series of samples taken in row 55. In this row, on June 22, 937 hookworm larvae were isolated from a pint of soil taken from a pollution spot 15 yards into the cane, and on July 1, 417 were found within the same pollution center. How definitely localized this center of soil infestation was is shown by the results of the examination of samples taken on both the above dates near the center. Samples 12, 64, 67, 13, 66, 14 and 62, taken from row 55, illustrate this point, since they show almost no general migration from this center. These specific instances, coupled with the general localized distribution of soil infestation in the cane, show that under the conditions in this strip of cane where everything appeared favorable for migrations, the infective hookworm larvae stayed close to the point of development. There is some evidence that they were carried about by water, but on the whole soil infestation was very much localized. This finding was supported by a series of experiments by Augustine (1922*a*) which showed that under a variety of conditions infective hookworm larvae do not migrate to any appreciable distance.

Certain data were obtained from examination of the soil samples from the strip of cane listed above, which indicated that infective hookworm larvae die out rather quickly in the soil. While the numbers of larvae found in the samples were quite large it struck us early in the work that if the infective hookworm larvae could live for a year or more in the soil, that with the gross daily pollution of this field, by individuals heavily infested with hookworm, the numbers of larvae present at these polluted spots would be exceedingly large.

Data from two particular centers of soil pollution also support this view. For some reason in June and early July there was an appreciable shifting of soil pollution away from the area in the cane, including rows 29, 30 and 31. This has been noted earlier in the section on soil pollution. Our records show that this reduction in pollution was quickly reflected in a reduction in numbers of larvae isolated from samples taken from this area. This is brought out by a comparison of the samples taken from row 30 on June 24 and 27 with the series taken from the same row on July 13. The twelve samples taken at the earlier dates gave 100 per cent. positives with a total of 213 larvae, or 19.2 larvae per sample. The seven samples taken

TABLE 7.

From drains of cane field strip.

o.	Date taken.	Location.	Hookworm larvae found.		
			No. sheathed.	No. unsheathed.	Total.
	July 4	D. 1; 8	..	..	0
	" 4	D. 1; 17	4	..	4
	" 4	D. 1; 25	..	..	0
	" 4	D. 1; 33	3	..	3
	" 6	D. 1; 42	..	1	1
	" 6	D. 1; 50	..	..	0
	June 24	D. 2; 1	..	..	0
	" 24	D. 2, 6	..	2	2
	July 13	D. 2; 8	1	1	2
	June 24	D. 2; 11	..	1	1
	" 24	D. 2; 16	..	3	3
	July 13	D. 2; 17	4	..	4
	June 24	D. 2; 21	2	19	21
	July 13	D. 2; 25	..	..	0
	June 24	D. 2; 26	..	6	6
	" 24	D. 2; 31	..	..	0
	July 13	D. 2; 33	..	1	1
	June 27	D. 2; 33	9	..	9
	" 27	D. 2; 42	2	..	2
	" 27	D. 2; 50	5	..	5
	" 27	D. 2: 55	..	..	0
	" 29	D. 2; 67	1	9	10
	" 29	D. 2: 75	..	..	0
	July 1	D. 2, 83	..	..	0
	" 1	D. 2; 92	1	..	1
	June 27	D. 3; 1	3	1	4
	July 13	D. 3; 5	1	..	1
	" 13	D. 3; 8	1	247	248
	June 27	D. 3; 8	24	13	37
	" 27	D. 3; 17	9	4	13
	July 13	D. 3; 17	3	20	23
	June 27	D. 3; 25	10	4	14
	July 13	D. 3; 25	2	..	2
	June 27	D. 3; 33	11	1	12
	July 13	D. 3; 33	..	3	3
	June 27	D. 3; 37	2	..	2
	" 27	D. 3; 40	1	3	4
	" 29	D. 3; 50	4	50	54
	July 1	D. 3; 50	6	21	27
	June 29	D. 3; 58	..	..	0
	" 29	D. 3; 67	..	..	0
	July 1	D. 3; 75	..	..	0
	" 1	D. 3; 83	1	..	1

TABLE 7—*Continued.*

Sample No.	Date taken.	Location.	Hookworm larvae found.		
			No. sheathed.	No. unsheathed.	Total.
72	July 1	D. 3; 165	..	..	0
108	" 6	D. 4; 8	16	19	35
109	" 6	D. 4; 17	..	..	0
110	" 6	D. 4; 25	..	..	0
111	" 6	D. 4; 33	..	..	0
112	" 6	D. 4; 42	..	..	0
113	" 6	D. 4; 50	..	..	0
114	" 6	D. 5; 5	1	32	33
187	July 13	D. 5; 5	..	8	8
96	" 4	D. 5; 8	2	85	87
188	" 13	D. 5; 9	...	1	1
115	" 6	D. 5; 12	4	7	11
189	" 13	D. 5; 13	2	..	2
97	" 4	D. 5; 17	..	..	0
98	" 4	D. 5: 25	..	..	0
99	" 4	D. 5; 33	..	1	1
116	" 6	D. 5; 42	..	..	0
117	" 6	D. 5; 50	..	..	0

on July 13 gave five positives with a total of 45 larvae, or an average of 9 larvae per sample. More striking still in this connection are the findings from the samples taken in row 55. A fresh stool and evidences of former pollution were found at a place about fifteen yards from the edge of this row on June 22. Although this place was inspected on each subsequent visit to the area, no further evidence of pollution was found. The results from samples 11, 65, 10 and 64, taken from row 55, show a very intense center of soil infestation present at this place on June 22 and July 1. Sample No. 186, taken on July 13, gave only nine larvae, and subsequent samples from this place soon showed a complete dying out. The sample of July 13 was taken over the whole area of former soil infestation from about 14½ to 15½ yards down this row, and in our estimation gives a fair test of the reduction of soil infestation. Since the last pollution recorded in this spot was on June 22 this record shows an enormous reduction in soil infestation in a period of only about three weeks. These observations on shortness of life of infective hookworm larvae led to the planning and carrying out of a more comprehensive field experiment, to test in this strip of cane the reduction in soil infestation which follows only occasional and not comparable to that in the cane strip. It seems to us, therefore, that the chief cause of the heavy infestation of the people of this area with hookworms was the habit which many of them had of visiting certain spots near the edge of the cane field for the purpose of defecation.

EXPERIMENTAL STUDY OF THE REDUCTION OF SOIL INFESTATION IN THE CANE FIELD.

The data from the original examinations of soil samples, noted above, which indicated a rapid dying out of larvae in the soil, stimulated us to carry out a field experiment along this line. The strip of cane, west of the barracks, which had been studied so intensively for soil pollution and soil infestation, was chosen for this experiment. The plan of the experiment was, first to bring about a decrease in the number of hookworm eggs deposited on this area, and to make examinations of soil samples at intervals to determine the effect in reducing soil infestation. Two methods were used in reducing the deposit of hookworm eggs in this area. The first was the attempt to reduce soil pollution by the introduction of latrines and educational work, the successful results of which are recorded earlier in this paper. The other was by treatment to reduce the mass infestation of the people of this area and consequently to reduce their output of eggs. The reduction of the soil pollution began between July 18 and July 22 and there was never any return to the previous conditions (see Chart 3). The first treatments were started on July 22. There was therefore between July 22 and August 1 an enormous reduction in the numbers of eggs deposited in the strip of cane under consideration.

The next step was to work out some method by which it would be possible to measure the reduction of soil infestation. It was decided to select from the most heavily infested places in the strip of cane thirty-six spots for examination and to compare the findings from samples taken at intervals from these places. Table 8 gives the location of these spots chosen for examination. Each of the places between rows had an area of from two to four square yards and was staked off at the time when the first series of samples was taken. For the experimental places in the drains the stakes were set about three yards apart. In taking a sample from these places small bits of the surface were scraped off at intervals over the whole area. This method of taking samples in connection with the large size of the experimental areas made it certain that the taking of a single sample would not appreciably diminish the soil infestation of the area.

The first series of thirty-six samples from these areas was taken on July 13 and July 18, before the reduction in soil pollution started and before treatments had been instituted. The records of infective larvae from this series show a very intense soil infestation on this date (see Table 8). Of course it is impossible to know the age of the larvae at the various places where the infestation was found. It is perfectly evident that the larvae infesting the sum total of these areas would contain all ages, and only a comparatively small proportion of them would be newly developed at the time of the first examination. Especially would this be true in certain areas such as Rows 29, 30 and 31, on which, as noted earlier, there had been a considerable reduction in pollution prior to July 13. It is therefore evident that the rate of reduction in the numbers of the larvae from this experimental area would be more rapid than in an experiment in which the larvae would all be fresh from cultures at the start.

The second series of examinations, which was made on August 8 (see Table 8), a little less than three weeks after the reduction of pollution, gave most striking results. There was a reduction of about 93 per cent. in the larvae found, and a number of samples were negative. Especially significant is the fact that the findings from the samples from Rows 29, 30 and 31, where a reduction of pollution before July 13 had been noted, were all negative. The records from the examinations made on August 22 showed still further reductions in the numbers of larvae, and those of September 5, made about six weeks after the reduction of soil pollution, showed an almost complete dying out of the larvae, only seven larvae being isolated from the whole thirty-six samples. In other words in less than six weeks this whole strip of cane had by the practical elimination of the deposit of hookworm eggs been changed from a dangerous ground-itch spot to a place where it was difficult to find any infective hookworm larvae in the soil.

An examination of Table 8 shows that there was a more rapid reduction of larvae in the area south of drain 2. An examination of the pollution surveys (Charts 1 and 2) shows that even before the educational work there was a shifting of pollution away from this part of the field, and that in the later surveys most of the stools were deposited north of this line.

TABLE 8.

Data showing reduction of soil infestation in the cane field strip.

No.	Location.	Ex. I. July 13–18.			Ex. II. Aug. 8.			Ex. III. Aug. 22.			Ex. IV. Sept. 5.		
		S.	U.	Tot.	S.	U.	Tot.	S.	U.	Tot.	S.	U.	Tot.
190	Row 3; 33	16	66	82	17	40	57	...	13	13	...	...	0
192	" 6; 12	12	136	148	1	20	21	...	12	12	1	1	2
154	" 14; 4	1	36	37	...	2	2	...	...	0	...	...	0
155	" 14; 7	2	9	11	1	2	3	...	...	0	3	...	3
156	" 15; 6	72	21	93	...	1	1	...	3	3	...	...	0
157	" 15; 9	7	68	75	...	4	4	...	2	2	...	...	0
194	" 15; 13	......	4	4	6	2	8	...	1	1	...	...	0
195	" 16; 7	1	2	3	1	3	4	1	4	5	...	...	0
196	" 20; 10	18	10	28	...	...	0	...	...	0	...	...	0
197	" 23; 7	8	13	21	...	1	1	4	3	7	...	...	0
198	" 25; 10	......	35	35	2	3	5	1	9	10	...	1	1
199	" 25; 5	2	1	3	...	3	3	...	...	0	...	...	0
200	" 26; 7	65	52	117	2	2	4	...	...	0	...	1	1
202	" 28; 5	3	14	17	5	3	8	...	...	0	...	...	0
203	" 28; 10	35	5	40	...	...	0	1	...	1	...	...	0
158	" 29; 8	2	14	16	...	...	0	...	...	0	...	...	0
159	" 30; 1	......	2	2	...	...	0	...	...	0	...	...	0
162	" 30; 10	......	8	8	...	...	0	...	2	2	...	...	0
164	" 30; 22	29	4	33	...	...	0	...	...	0	...	...	0
166	" 31; 5	5	......	5	...	...	0	...	...	0	...	...	0
167	" 35; 4	20	......	20	1	...	1	...	...	0	...	...	0
168	" 35; 9	2	15	17	...	4	4	...	...	0	...	...	0
204	" 37; 12	161	150	311	...	...	3	...	...	0	...	...	0
186	" 55; 15	......	9	9	...	2	2	...	...	0	...	...	0
172	Dr. 2; 8	1	1	2	...	...	0	...	...	0	...	...	0
173	" 2; 17	4	......	4	...	1	1	...	...	0	...	...	0
174	" 2; 25	......	......	0	...	...	0	...	...	0	...	...	0
175	" 2; 33	......	1	1	...	...	0	...	...	0	...	...	0
176	" 3; 5	1	......	1	1	1	2	...	1	1	...	...	0
177	" 3; 8	1	247	248	...	1	1	...	...	0	...	...	0
178	" 3; 17	3	20	23	...	1	1	...	16	16	...	...	0
179	" 3; 25	2	......	2	...	...	0	...	...	0	...	...	0
180	" 3; 33	......	3	3	...	3	3	...	1	1	...	...	0
187	" 5; 5	......	8	8	...	...	0	...	...	0	...	...	0
188	" 5; 9	......	1	1	...	...	0	...	...	0	...	...	0
189	" 5; 13	2	......	2	...	...	0	...	...	0	...	...	0
Total	Drains	14	281	295	1	6	8	...	18	18	...	...	0
Total	In Rows	461	674	1,135	36	92	128	7	49	56	4	3	7
	Total	475	955	1,430	37	98	136	7	67	74	4	3	7

The reduction in the numbers of larvae in the drains is especially significant since they reflect the general soil infestation of the area which they drain. The first series, from drains of the experimental area, was taken on July 13. Already the results from drains 2 and 5 showed some reduction over previous examinations. The results from drain 3 were especially striking. Up to and including July 13 fourteen samples, all positive, had been taken from the first 50 yards along this drain, with the high average of 32 larvae per sample. The rapid and complete reduction in the numbers of larvae from this drain is shown in Table 8. These particular instances and the general and widespread dying out of the infective hookworm larvae from this strip of cane field show that it was only a matter of six weeks after the elimination of soil pollution until the infestation of the soil practically died out.

This evidence on the shortness of life of infective hookworm larvae in the soil under tropical conditions is supported by the findings from another field exeriment, under quite different conditions (Cort and Payne, 1922*b*), and a series of laboratory experiments (Augustine, 1922*c*). So that the conclusion can be drawn that under tropical conditions, where the high temperatures are favorable to great activity of the hookworm larvae, their life in the soil is quite short.

SUMMARY.

1. An intensive epidemiologic study was made of an area with a high incidence of hookworm disease, in a sugar estate in Trinidad, British West Indies, to determine the exact sources of human infestation and to learn the effect on human infestation, soil pollution and soil infestation of a control campaign.

2. Of the 142 people, both East Indians and negroes, examined from the area chosen for study, 117 were found to be infested with hookworms.

3. A series of three treatments greatly decreased both the number of positives and the total mass infestation of these people.

4. Soil pollution was widespread and very gross in this area, being especially concentrated at certain easily accessible places in the cane field, to the west of the barracks, where the people lived.

5. Surveys of the pollution in the cane field showed a great reduction after the building of an adequate number of latrines and the carrying on of an educational campaign.

TABLE 8.

Data showing reduction of soil infestation in the cane field strip.

No.	Location.	Ex. I. July 13-18.			Ex. II. Aug. 8.			Ex. III. Aug. 22.			Ex. IV. Sept. 5.		
		S.	U.	Tot.	S.	U.	Tot.	S.	U.	Tot.	S.	U.	Tot.
190	Row 3; 33	16	66	82	17	40	57	...	13	13	...	...	0
192	" 6; 12	12	136	148	1	20	21	...	12	12	1	1	2
154	" 14; 4	1	36	37	...	2	2	...	...	0	...	...	0
155	" 14; 7	2	9	11	1	2	3	...	...	0	3	...	3
156	" 15; 6	72	21	93	...	1	1	...	3	3	...	...	0
157	" 15; 9	7	68	75	...	4	4	...	2	2	...	...	0
194	" 15; 13	......	4	4	6	2	8	...	1	1	...	...	0
195	" 16; 7	1	2	3	1	3	4	1	4	5	...	...	0
196	" 20; 10	18	10	28	...	...	0	...	...	0	...	...	0
197	" 23; 7	8	13	21	...	1	1	4	3	7	...	...	0
198	" 25; 10	......	35	35	2	3	5	1	9	10	...	1	1
199	" 25; 5	2	1	3	...	3	3	...	...	0	...	...	0
200	" 26; 7	65	52	117	2	2	4	...	...	0	...	1	1
202	" 28; 5	3	14	17	5	3	8	...	...	0	...	...	0
203	" 28; 10	35	5	40	...	...	0	1	...	1	...	...	0
158	" 29; 8	2	14	16	...	...	0	...	...	0	...	...	0
159	" 30; 1	......	2	2	...	...	0	...	...	0	...	...	0
162	" 30; 10	......	8	8	...	...	0	...	2	2	...	...	0
164	" 30; 22	29	4	33	...	...	0	...	...	0	...	...	0
166	" 31; 5	5	......	5	...	...	0	...	...	0	...	...	0
167	" 35; 4	20	......	20	1	...	1	...	...	0	...	...	0
168	" 35; 9	2	15	17	...	4	4	...	...	0	...	...	0
204	" 37; 12	161	150	311	...	...	3	...	...	0	...	...	0
186	" 55; 15	......	9	9	...	2	2	...	...	0	...	...	0
172	Dr. 2; 8	1	1	2	...	...	0	...	...	0	...	...	0
173	" 2; 17	4	......	4	...	1	1	...	...	0	...	...	0
174	" 2; 25	......	......	0	...	...	0	...	...	0	...	...	0
175	" 2; 33	......	1	1	...	...	0	...	...	0	...	...	0
176	" 3; 5	1	......	1	1	1	2	...	1	1	...	...	0
177	" 3; 8	1	247	248	...	1	1	...	...	0	...	...	0
178	" 3; 17	3	20	23	...	1	1	...	16	16	...	...	0
179	" 3; 25	2	......	2	...	...	0	...	...	0	...	...	0
180	" 3; 33	......	3	3	...	3	3	...	1	1	...	...	0
187	" 5; 5	......	8	8	...	...	0	...	...	0	...	...	0
188	" 5; 9	......	1	1	...	...	0	...	...	0	...	...	0
189	" 5; 13	2	......	2	...	...	0	...	...	0	...	...	0
Total	Drains	14	281	295	1	6	8	...	18	18	...	...	0
Total	In Rows	461	674	1,135	36	92	128	7	49	56	4	3	7
	Total	475	955	1,430	37	98	136	7	67	74	4	3	7

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. VII.

AN EPIDEMIOLOGIC STUDY OF HOOKWORM DISEASE IN A CACAO ESTATE.*

BY WILLIAM W. CORT, PH.D., AND GEORGE C. PAYNE, M.D.

(Received for publication December 13, 1921.)

INTRODUCTION.

Since the production of cocoa is one of the chief industries of Trindad, British West Indies, it was of interest during our investigation there to study the conditions influencing the spread of hookworm disease on a cacao estate. The objects of this study were two-fold, viz.: (1) to locate the sources of human infestation and (2) to determine the reduction of soil infestation produced in localized centers of pollution in a cacao grove, after the treatment of the people who visited these spots.

DESCRIPTION OF AREA.

For this study a part of an estate was chosen which was located about five miles from our laboratory. This area included two barracks and one detached dwelling, the yards surrounding the houses and that part of the cacao grove immediately back of the two barracks (see map). The cacao grove, which was thickly planted with full-grown trees and shaded by large immortel trees, was easily entered by two paths from the barracks. The detached house which was the home of the negro driver, his wife and five children, was in good repair, but the two barracks were quite dilapidated. The barracks were inhabited by East Indian laborers, of the same type as those on the sugar estate described in number six of this series (Cort and Payne, 1922*a*). In Barrack 1 lived five adult East Indians, one male and four females, and six children, while in Barrack 2 there lived an East Indian, his wife and four children. All these people had lived on this estate from four to seven years. The only animals on

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* This paper is the seventh of the series on the hookworm investigations of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

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There was an open-backed latrine back of Barrack 2, which was in filthy condition at the time of our early visits to this area (between June 6 and June 10). At this time it was evidently used by a few people, but later visits showed that its use had been entirely abandoned. Since this was the only latrine available to the people of the two barracks, it was evident that soil pollution was the rule.

Very little soil pollution was found near these two barracks. Some scattered stools were found at later visits around a banana tree, back of Barrack 1, and another pollution spot was found where the path from Barrack 2 entered the cacao. Inquiries brought out the statement that these places were visited only by children. It was also learned that the people did not wash themselves near the houses

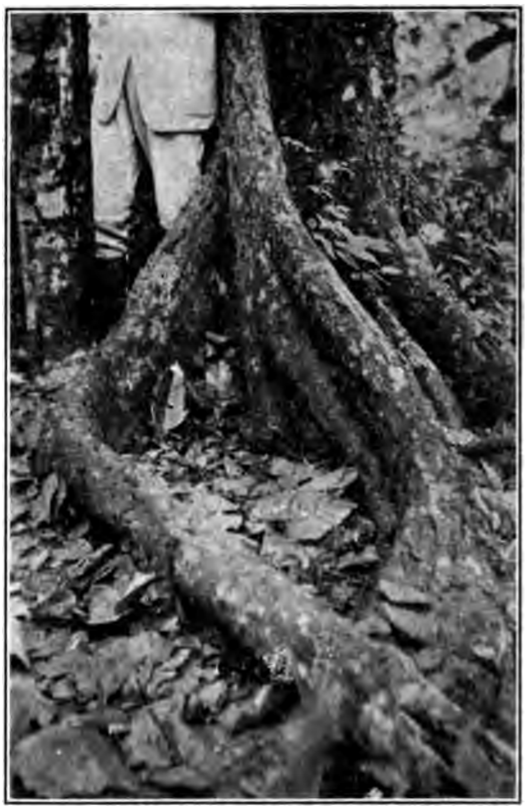

FIG. 2. A " natural latrine " entirely enclosed by the roots of an immortel tree (P_2).

a were a pig, kept by the negro family, and a small number
kens which ranged over the whole territory, around the houses
ck into the cacao.

HUMAN INFESTATION.

re were only two light cases of hookworm disease in the negro
the father and the eight-year-old son. Fifteen of the seven-
ast Indians in the two barracks were infested with hookworms,
gatives being a girl of three and a boy of twelve. The infesta-
vere heavy, except in the young children, and some showed
t clinical symptoms. The people of this area were given three
ents by the method described in our previous paper (Cort and
1922a, p. 113), between July 23 and August 6. Only two
positives, both East Indian women, escaped treatment, one
cealing herself and the other on account of an advanced preg-
Of the fifteen individuals treated, re-examinations were made
en, of which eight were still infested. From what is known of
cts of similar treatments elsewhere it can be argued that there
great reduction of mass infestation following the treatments.

CONDITIONS OF SOIL POLLUTION.

evidence of soil pollution was found around the house where
gro family lived. There was a well-constructed pit latrine near
use, which was kept in good condition, and used by all the
rs of the family.

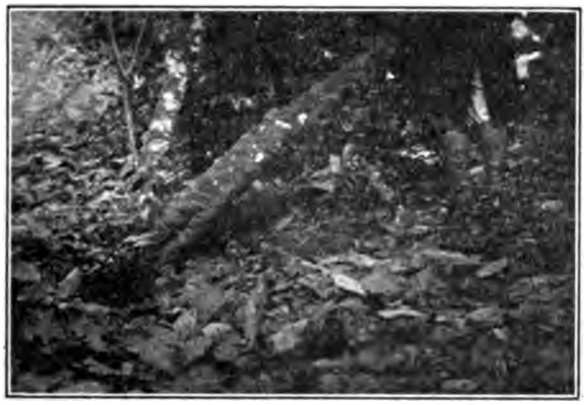

FIG. 1. A heavily polluted spot beside a leaning cacao tree (P_1).

There was an open-backed latrine back of Barrack 2, which was in filthy condition at the time of our early visits to this area (between June 6 and June 10). At this time it was evidently used by a few people, but later visits showed that its use had been entirely abandoned. Since this was the only latrine available to the people of the two barracks, it was evident that soil pollution was the rule.

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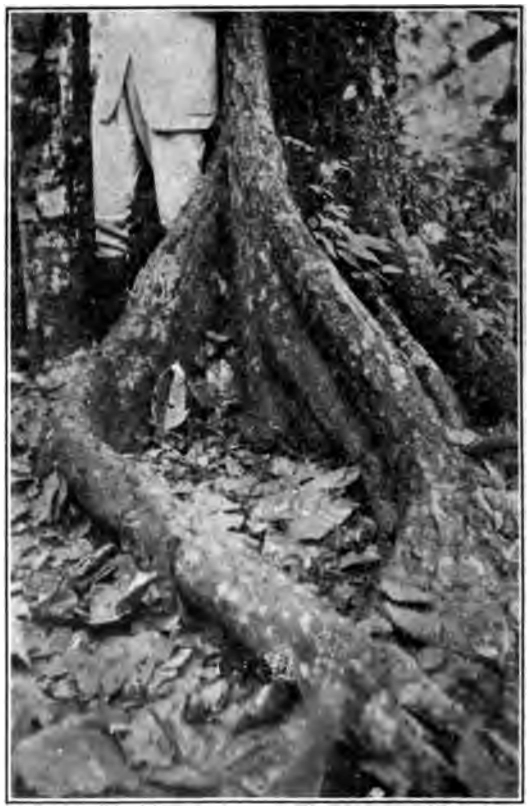

FIG. 2. A "natural latrine" entirely enclosed by the roots of an immortel tree (P_2).

fter defecation, but carried bottles of water out with them into the acao grove for this purpose.

In the cacao grove back of these two barracks there were definitely ocalized centers of pollution, which were visited day after day by the eople of the barracks. At the time of the first visits to this place June 4 to 20) there were found four of these pollution spots which onstituted, as it were, natural latrines. The location of these pollu-ion centers, which will be designated as P_1, P_2, P_3 and P_4, can be seen n the map. The spot designated as P_1 (Fig. 1) was at the side of a eaning cacao tree; P_2 (Fig. 2) was an area enclosed by the roots of a arge immortel tree; P_3 (Fig. 3) was near P_2 at the side of the exposed oots of a cacao tree and P_4 (Fig. 4) was the area between the buttress oots of a second immortel tree. During the first series of surveys here was evidence of daily pollution at all these places. As in the pol-uted areas of the sugar cane field (Cort and Payne, 1922, p. 119), the tools disappeared quickly, chiefly from the action of the rain and the ctivities of insects, so that the places did not become foul smelling nd their use seemed to the people much preferable to the use of the lthy latrine. That these "natural latrines" were in constant use s evidenced by the finding of pollution in them on visits made July 20 and 26 and later on September 3. At these later visits two new ollution centers were found around the roots of immortel trees

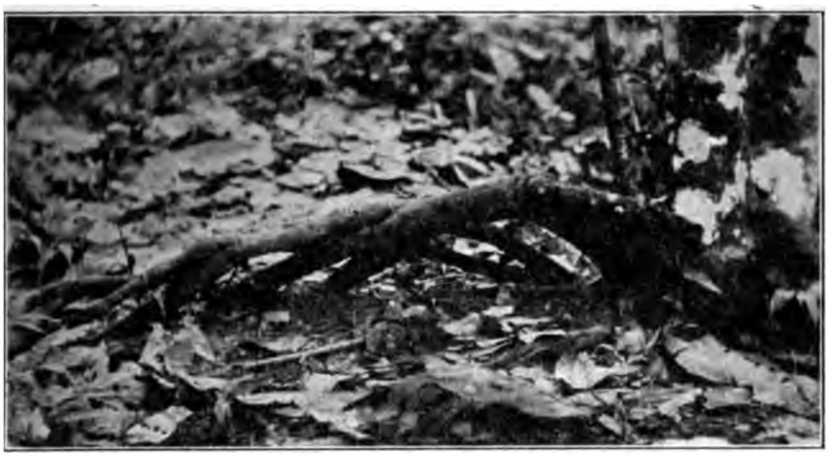

FIG. 3. A concentrated pollution center at the side of the exposed roots of a cacao tree (P_3).

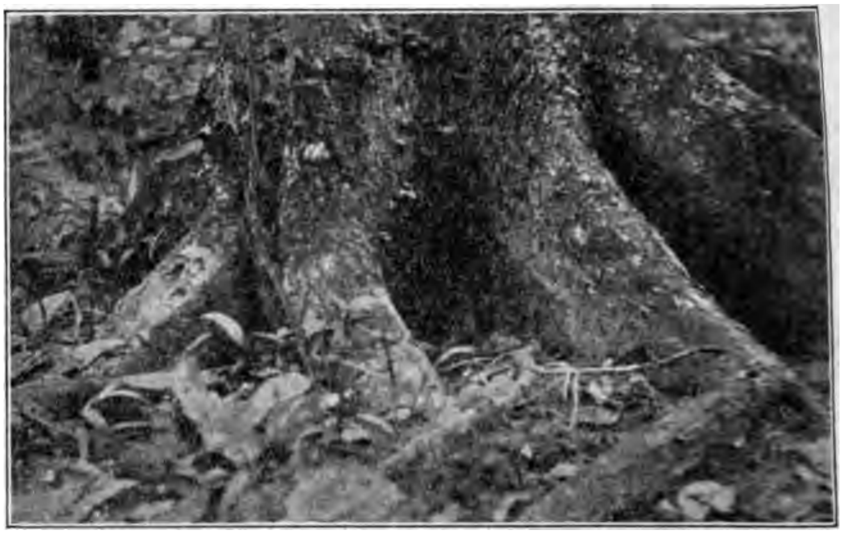

FIG. 4. A polluted spot between the buttress roots of an immortel tree (P_4).

(see P_5 and P_6 on the map). At P_5 the pollution was so great that there was formed between two of the roots a small area of liquified sludge, like that found in pit latrines, in which fly larvae were working. The pollution at these spots in the cacao was so definitely localized and they were so constantly visited that they may be considered as natural latrines, which served the people of these two barracks, and which seemed to them much cleaner and less objectionable than the ordinary pit latrines.

SOIL INFESTATION.

The study of soil infestation in this area was made by the use of the isolation apparatus (Cort, Ackert et al., 1921) in much the same way as in the investigation of the sugar estate previously described (Cort and Payne, 1922*a*). On June 13 and 17 a series of 12 soil samples were taken from immediately around the houses, from wet spots where the people washed themselves and their utensils, and from the drains. The results of the examination of all these samples for hookworm larvae were negative. About a month later seven more soil samples were taken from the same situations. Only one of these, a sample from a drain at Barrack 1, was positive, giving only one hookworm larva. The locations from which these nineteen soil samples were taken is shown on the map.

The other soil samples which were taken from this area, outside the cacao grove, are shown on the map. They were taken with relation to known soil pollution and show that centers of slight soil infestation had developed in the area polluted by the children, near the banana tree back of Barrack 1 and around the latrine.

Fifty soil samples were examined from the cacao grove back of Barracks 1 and 2, which were taken with relation to the pollution spots (P_1 to P_6). The surface of the ground in the cacao grove offered three different conditions, viz.: (1) bare clay soil, (2) clay covered by a layer of humus, and (3) that with the surface covered with a carpet of twigs and dead leaves (see Figs. 1 to 4). At first in taking the soil samples the surface leaves were brushed aside, but later it was found that the infective hookworm larvae were often found on the surface of the leaves, especially when they were wet with dew or rain (see Augustine, 1922*b*, p. 175). After this finding the soil samples were taken directly from the surface and consisted either of leaves or soil. Table 1 gives a complete list of samples taken from this environment.

TABLE 1.

Record of examination of soil samples from the cacao grove back of barracks 1 and 2. A. Leaning cacao tree (P_1).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Unsheathed.	Total.
28	June 13	At pollution center...	Surface humus.....	..	..	4
29	" 13	5 ft. W. of pollution center...........	Surface humus.....	..	..	0
38	" 15	At pollution center...	Leaves and humus .	12	16	28
40	" 15	At pollution center...	Surface clay and humus.........	..	..	0
63	" 17	At pollution center...	Leaves only.......	26	7	33
64	July 20	At pollution center...	Humus...........	96	30	124
65	" 20	1 yd. W. of pollution center...........	Leaves and humus .	24	4	28
66	" 20	1 yd. N. of pollution center...........	Leaves and humus .	26	8	34
67	" 20	Leaves around edge of cleared pollution center...........	Leaves...........	345	25	370
100	" 26	2 yds. W. of pollution center...........	Leaves and humus .	3	20	23
101	" 26	4 yds. W. of pollution center...........	Leaves and humus .	..	..	0

B. Root enclosure at largest immortel tree (P_2).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Un-sheathed.	Total.
3	May 31	At pollution center within roots.......	Surface clay and humus..........	..	..	8
30	June 13	At pollution center within roots.......	Surface clay and humus..........	..	..	24
31	" 13	2 ft. S. of pollution center............	Humus...........	..	..	0
37	" 15	At pollution center...	Leaves............	..	..	1
42	" 15	" " " ...	Moist twigs.......	..	..	147
61	" 17	" " " ...	Leaves............	5	3	8
68	July 20	" " " ...	Surface clay, humus and leaves.......	1	2	3
69	" 20	In front of root just outside of pollution center............	Leaves and humus.	1	1	2
104	" 26	At pollution center...	Leaves and soil....	..	1	1

C. At foot of cacao tree, seven feet north of P_2 (P_3).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Un-sheathed.	Total.
32	June 13	At pollution center...	Surface soil........	..	..	3
33	" 13	At other side of tree, just out of pollution center............	Surface clay.......	..	..	0
62	" 17	At pollution center...	Leaves............	16	47	63
70	July 20	" " " ...	Leaves and humus.	19	14	33
71	" 20	2 yds. E. of pollution center............	Leaves, humus and clay............	..	..	0
105	" 26	At pollution center...	Leaves and humus.	1082	28	1110

While a large number of infective hookworm larvae were found the numbers were not in proportion to the concentration of the pollution. The finding of large numbers of unsheathed infective hookworm larvae in these samples need not be discussed here, since this point was covered in an earlier number of this series. (Cort, Augustine et al., 1922.)

D. At base of immortel tree, 25 feet northwest of P_2 (P_4).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Unsheathed.	Total.
34	June 13	At pollution center	Surface clay	.	..	0
35	“ 13	“ “ “	“ “	..	..	1
43	“ 15	“ “ “	Leaves and humus	..	..	0
72	July 20	“ “ “	“ “ “	1	1	2
73	“ 20	At base of small cacao, two yards east of pollution center	“ “ “	..	..	0
74	“ 20	At edge of drain, 1 yard from pollution center	“ “ “	..	..	0
75	“ 20	At head of small drain, 1½ yards from pollution center	“ “ “	2	1	3
76	“ 20	3 yards down drain	“ “ “	..	..	0
77	“ 20	4 yards down drain	“ “ “	..	..	0
78	“ 20	6 yards down drain	“ “ “	..	..	0
79	“ 20	9 yards down drain	“ “ “	..	..	0
80	“ 20	2 yards south of No. 73	“ “ “	..	..	0
81	“ 20	3 yards southwest of No. 73	“ “ “	..	..	0
106	“ 26	At pollution centers	“ “ “	5	15	20
107	“ 26	Same as No. 73	“ “ “	..	4	4

The results of the examinations of the soil samples from the neighborhood of the leaning cacao tree (P_1) (Fig. 1) are given in Table 1, *A*. As was expected from the constant pollution at this place, there was found a high percentage of positives with numerous hookworm larvae. Some distribution of larvae out from the pollution center is indicated by the findings from samples 65, 66, 67 and 100. But that such soil infestation was not widespread is shown from samples 29 and 101. The slight spreading of the larvae from this pollution center could be easily accounted for by the scratching of chickens which frequently visited this place.

The results of the examinations of the samples from P_2 (Table 1, *B*) were very surprising, when it is considered that in an area about six feet long and two feet wide, enclosed by buttress roots

(Fig. 2), stools were apparently deposited daily by two or three people, at least one of whom was known to be heavily infested with hookworms. The soil within this area succeeded in absorbing the pollution without the formation of sludge. That such few larvae were isolated from the soil of this place indicates that for some reason or other the conditions here were not favorable for the development of hookworm larvae.

The findings from P_3 (Fig. 3) which are given in Table 1, *C*, indicate the development of intense soil infestation both on the leaves and the surface of the soil. The results of the examination of sample 71 show that there is very little migration under the conditions in the cacao, even from centers of intense soil infestation.

E. At base of immortel tree, 22 feet north of P_1 (P_5).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Un-sheathed.	Total.
82	July 20	2 yds. in front of pollution center......	Leaves and humus.	..	..	0
83	" 20	At pollution spot....	" " " .	1	3	4
84	" 20	At edge of drain 1 yd. east of pollution spot............	" " " .	..	..	0
85	" 20	At edge of drain 1 yd. north of pollution spot............	" " " .	..	..	0
86	" 20	In drain 1½ yards east of pollution spot...	Clay..............	..	..	0
87	" 20	2 yds. west of pollution spot.........	Leaves and humus.	..	3	3
102	" 26	At pollution spot....	" " " .	2	3	5

F. At base of immortel tree, 30 feet north of P_1 (P_6).

No.	Date.	Location of sample.	Character of sample.	Larvae found.		
				Sheathed.	Un-sheathed.	Total.
103	July 26	At pollution center...	Leaves and humus.	135	106	241

The data from the examination of the soil samples taken from areas P_4 and P_5 (Table 1, *D* and *E*), indicate, either conditions unfavorable for the development of hookworm larvae, or pollution by negative or lightly infested individuals. The results from sample 103 (Table 1, *F*) show that a center of soil infestation had developed at P_6.

The results of the examination of soil samples in the cacao (Table 1) show that centers of soil infestation had developed at certain polluted spots. These results are surprisingly irregular, when the constant pollution of these places, and the heavy infestation with hookworms of the people visiting them are considered. It is impossible to explain these irregularities from the data at hand. Three possible factors suggest themselves which might be responsible, viz.: (1) the presence of leaves on the soil which complicated the taking of the soil samples; (2) possible variations in the degree of infestation of people visiting the different pollution centers; and (3) the development at certain of these places of conditions unfavorable to the development of hookworm larvae. Certain activities of the infective hookworm larvae in the cacao environment have been studied by Augustine (1922, p. 175). His findings that the larvae do not migrate any distance under conditions existing in the cacao grove and that they have a tendency to get onto the upper surfaces of the leaves were verified by our field studies.

SOURCES OF HUMAN INFESTATION.

The data given above indicate that the only really important sources of infestation with hookworms in this area are the centers of soil infestation developed at the highly polluted spots in the cacao. The area around the banana tree, back of Barrack 1, might have been responsible for some infestation, especially in children, and the slight soil infestation in front of the latrine might have given a few worms to those visiting it. However, the soil infestation at the polluted centers back of the barracks, the constant visiting of these places by the East Indians, and the fact that this part of the cacao was known as a ground itch area, suggests that from here most of the hookworm infestation found in Barracks 1 and 2 was derived. Further evidence for this view is given by the finding that all the negro family were negative, except the driver and his eight-year-old son. It was found by inquiry that the negro boy had acquired ground itch from playing in the cacao back of the East Indian barracks, and that the driver, who wore heavy boots, had had ground itch on his hands from working in this area. Therefore, here again as in the sugar estate (Cort and Payne, 1922, p. 142) it was found that the soil pollution habits of the people were chiefly responsible for their hookworm infestation, especially when they were accustomed to visit the same place day after day.

REDUCTION OF SOIL INFESTATION FOLLOWING TREATMENT.

We decided to try out in this area the reduction of soil infestation produced by three routine treatments, when there had been no reduction of soil pollution. The records of treatments given above show that two people were untreated and that of the eleven examined after treatment eight were still positive. Therefore, while a considerable reduction of mass infestation must have resulted from the treatments, there was still left an appreciable infestation of the majority of those treated. Table 2 shows by a comparison of the results of the examination of soil samples, taken July 20 and 26, from the centers of pollution in the cacao, with a series from the same places taken September 3, that this reduction of mass infestation was quickly reflected in the reduction of soil infestation. The samples of September 3 were taken just after a rain and consisted of the scrapings from the entire surface of the polluted spots. Since the larvae have been shown to come to the surface after it is moistened (Augustine,

TABLE 2.

Comparison of samples from polluted spots in the cacao grove (P_1–P_6), before and after treatment.

Sample No.	Location.	Ex. July 20 and July 26.			Ex. Sept. 3d.		
		Sheathed.	Unsheathed.	Total.	Sheathed.	Unsheathed.	Total.
64	P1. Cleared place at center	96	30	126	2	19	21
67	P1. Leaves around cleared place	345	25	370	1	9	10
105	P3	1,082	28	1,110	26	23	49
106	P4	5	15	20	..	..	0
102	P5	2	3	5	..	..	0
103	P6	135	106	241	1	8	9
Total....		1,665	207	1,872	30	59	89

1922*b*) these samples were taken under the most favorable conditions possible for locating infective hookworm larvae. This fact makes the great reduction in numbers of larvae shown by Table 2, all the more significant. These findings show that in areas of soil infestation in the cacao cultivation the length of life of infective hookworm larvae is short.

SUMMARY.

1. Examination of the people living in three houses on a cacao estate showed a heavy infestation with hookworms.

2. Soil pollution in this area was almost entirely restricted to definite spots—" natural latrines " in the cacao grove near the barracks.

3. The examination of soil samples showed very little soil infestation anywhere in the area, except at the " natural latrines " in the cacao, and the conclusion can be drawn that almost all the human infestation must have come from the habit of polluting the soil of the cacao.

4. Even in the "natural latrines" the findings were somewhat irregular, indicating the conditions were not always favorable for the development of hookworm larvae, and that they did not migrate from the place of development.

5. Examinations of the soil of the intensely polluted spots in the cacao, six weeks after three routine treatments had been given to the people, showed a very marked reduction of soil infestation, indicating that in this situation the life of the infective hookworm larvae is short.

For bibliography see No. VI of this series.

INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. VIII.

EXPERIMENTS ON THE MIGRATION OF HOOKWORM LARVAE IN SOILS.*

BY DONALD L. AUGUSTINE, B.S.

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HISTORICAL ACCOUNT.

Until Baermann (1917) devised an apparatus with which hookworm larvae could be successfully isolated from the soil, little or nothing was known of their activities under natural conditions. Throughout the literature, one is impressed with the idea that the infective hookworm larvae may actively migrate for considerable distances, and may thus give rise to infestation far from the place where the eggs were originally deposited.

Claude A. Smith (1903, p. 710) recorded that the larvae dart through a sandy soil at a lively rate for a few minutes to half an hour or more, and then straighten out and lie perfectly still for a corresponding length of time. In the report of the Porto Rico Anemia Commission (1904, p. 121) the statement was made that rain causes a distribution of larvae, and that this, together with their natural motility, may spread nests of soil infestation over a considerable area. Nicoll (1917, p. 163) in his discussion of the hookworm larvae, under natural conditions, made the following statement:

"The larvae now measure about 0.6 mm. in length and are very actively motile. Unless they have been washed away previously from the faeces, they now proceed to migrate to a more suitable locality, namely: the nearest water pool or patch of moist ground. Provided even a trace of moisture be present, they are capable of traversing considerable distances and may thus give rise to infection far from the place, where the faeces were originally deposited. How far and how rapidly they may wander in this fashion is not definitely known, but judging from laboratory experiences their rate is probably not less than five feet per hour, so that in the course of 24 hours, they may have wandered 40 yards. This means that an area of 5,000 square yards might become infected within 24 hours after the larvae started to migrate, i.e., within a week after the faeces were deposited."

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* This paper is the eighth of the series on the hookworm investigations of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

Chandler (1918, p. 259) pointed out that continual traveling in a straight line would probably never occur, but that a single stool containing hookworm eggs could easily infest at least several square yards. Similar statements on the spread of hookworm larvae were also made by Ozzard (1909, p. 779).

The first intimation that the infective hookworm larvae might be limited in their distribution throughout the soil was made by Price (1910, p. 205). He stated that in his opinion sub-soil pollution was not an important factor in hookworm disease, because it appears inconceivable that hookworm larvae will pass for any considerable distance through a sand and gravel sub-soil.

Based on the work of these authors, the opinion has become current that the infective hookworm larvae move out from centers of infestation and spread themselves over wide areas. This conception is founded largely upon the study of the behavior of the encapsuled larvae when mounted in water, or water and soil, on glass slides or in watch glasses, and examined microscopically. To what extent the migrations of the larvae occur from definite infested centers in soils of various types and under varying conditions, has not yet been demonstrated. The experiments which will be described in this paper were carried out in Trinidad, British West Indies, from May to September, 1921, in order to gain information on this very subject.

I wish here to express my appreciation to Dr. W. W. Cort, under whose direction the investigations embodied in this and the two following papers were carried on, for his constant interest and helpful suggestions. Thanks are also due Dr. James E. Ackert, of the Kansas State Agricultural College, Dr. Florence King Payne and Dr. George C. Payne, Trinidad, for assistance in securing material and for suggestions during the course of the work.

MATERIAL AND METHODS.

The larvae used in these experiments were obtained from cultures 5 to 8 days old. A detailed account of the method used in the preparation of the cultures is given in number two of this series (Cort, et al., 1922, p. 6). The larvae were carefully counted under the microscope, the 16 mm. objective being used, when the hookworm larvae were mixed with other nematodes, and a lower power, when they were in pure culture. As *Ancylostoma duodenale* is very rare in Trinidad it may be assumed that the larvae obtained were those of *Necator americanus.*

Two sizes of pans were used in the experiments on migration, viz.: (1) round tin dripping pans, 13 inches in diameter and 4 inches high, and (2) pudding pans 6 inches in diameter and 2 inches high.

The soils in which these larvae were studied were selected from the district in which these experiments were carried on, the Naparima District, which is in the south central part of Trinidad. They were chosen from areas free from soil pollution, and tested in the laboratory with the isolating apparatus for the presence of hookworm larvae and free-living nematodes. None of the samples were found to contain hookworm larvae, and where free-living nematodes were numerous, the soil was heated to 150° F. This temperature was found to be sufficient to kill all living organisms present without altering the composition of the soil itself.

The migrations of the hookworm larvae were studied in five distinct types of soils. Type 1 was a black, rather heavy, clay loam which contained no small stones. Type 2 was identical with type 1, except that it contained a greater amount of humus and was lighter. These two soils are classified by Williams (1921, pp. 106–109) under the Maparina Marls or the Black Soils of the Maparimas. Type 3 was also a heavy clay loam distinguished by its red color. It differed from types 1 and 2, chiefly in its chemical rather than mechanical composition being deficient in lime and having a high percentage of iron. Type 4 was a fine sand and clay mixture, light when dry or slightly moist, but heavy and compact when wet. Type 5 was a loose reddish sand, containing only a small amount of clay.

EXPERIMENTS ON ACTIVE MIGRATION.

Table 1 gives the data from a series of twenty experiments to test the active migration of infective hookworm larvae under a variety of conditions. In all the experiments, summarized in this table, the soils were placed to the depth of 1 cm. in the 13-inch dripping pans. The center was then determined, marked by a cord circle 1 inch in diameter, and the larvae placed within this circle. This area was termed Zone 1, or the center of infestation. When I wished to close an experiment the soil was further divided into Zones 2, 3 and 4. This was accomplished with an apparatus similar to a doughnut or cooky cutter. It consisted of three stationary tin bands of sufficient circumference to make each of these zones two inches wide. The soils of the three outer zones were transferred separately to units of the large isolation apparatus, without withdrawing the "cutter." The danger of mixing the soil of one zone with the next was thereby reduced to a minimum. Two units of the isolation apparatus were used for Zone 4, to keep the amount of soil in each unit approximately equal. The cutter was then removed, and the soil from the infestation center transferred to a unit of the small isolation apparatus.

In describing the moisture content of the soils the term "moist" indicates a soil which had a slightly sensible wetness, while the term "saturated" denotes a soil which had been thoroughly soaked and its surface fairly covered with water.

It will be seen from an examination of Table 1, that the larvae did not manifest the migratory habit which has been attributed to them, since in no instance were they found as far as four inches from the center in which they were placed. In only 4 experiments (Table 1, nos. 5, 7, 17 and 19) were larvae recovered from the third zone, and in each of these cases less than 2 per cent. of the total number of larvae recovered had reached this zone. It is also apparent that neither the type of soil nor the presence of vegetation enter as factors in the extent of active migration of hookworm larvae. A thick grassy sod was not available at the time of these experiments, and in order to study the larvae under this environment the soil—clay loam—was first heavily sown with a mixture of oats and wheat. The larvae were not added to the infestation centers until the grass had formed a heavy root system, and had reached a height of 3 to 4 inches. No perceptible difference was observed in the extent of migration of the hookworm larvae, when exposed to direct sunlight under temperatures varying from 93° F. to 101° F. (Experiment 20, Table 1). The migration pan was set out in direct sunlight during the day and returned to the laboratory at night. At no time was the soil allowed to become dry. As soon as a dry crust appeared on the surface it was evenly sprinkled with water, thus keeping it as uniformly "moist" as possible. While the sunlight had no apparent influence on the extent of spread of the larvae away from the infestation center, it had an indirect effect on the activities of the larvae within that area which is discussed in number eight of this series.

TABLE 1.

Experiments on the active migration of hookworm larvae. All these tests were made in the thirteen-inch pans.

Experiment number.	No. larvae in center of infestations.	Type of soil.	Conditions.	Time larvae remained in soil.	No. of H.W. larvae recovered from			
					Zone 1.	Zone 2.	Zone 3.	Zone 4.
1	800	Clay loam	Moist, shade	15 hours	409	15	0	0
2	1,000	Clay "	" "	16 "	583	274	0	0
3	1,800	Loose loam	" "	40 "	1,395	128	0	0
4	1,000	Loose "	" "	15 "	460	406	0	0
5	1,000	Clay loam	" "	7 days	236	84	1	0
6	1,000	Clay "	" "	7 "	478	39	0	0
7	512	Clay "	" "	11 "	89	145	3	0
8	1,100	Clay "	" "	21 "	13	5	0	0
9	650	Clay "	" "	42 "	1	0	0	0
10	525	Red clay loam	" "	15 hours	363	69	0	0
11	500	" " "	" "	22 days	6	3	0	0
12	277	Clay loam sod	" "	7 "	60	10	0	0
13	392	" " "	" "	21 "	2	3	0	0
14	361	" " "	" "	30 "	1	1	0	0
15	426	" " "	" "	37 "	1	0	0	0
16	498	Sand	" "	10 "	51	40	0	0
17	451	Sand	" "	22 "	77	16	1	0
18	498	Sand	" "	29 "	51	40	0	0
19	1,300	Sand and clay	" "	8 "	199	174	5	0
20	500	Clay loam	Moist, direct sun	35 "	5	0	0	0

Early in these experiments it was noted that there occurred a remarkable reduction from the original number of larvae placed in the infestation centers, even after a week's time. It will be noted, from Table 1, that this reduction increased with the number of days the larvae remained in the soil. Here, as well as in other experiments made, a large percentage of the larvae recovered had lost their sheaths.

This experimental evidence on the failure of the hookworm larvae to migrate in the soil is supported by similar findings of Cort and Payne (1922*a*, p. 137), in their study on the sources of hookworm infestation in a cane field. Instead of finding a uniform distribution of the larvae over the total area studied, they were found to be definitely localized at the places of soil pollution. Often where two soil samples were taken close together one would contain a large number of larvae, while the other would contain only a few or be even negative. As a specific example, approximately 1,000 hookworm larvae were isolated from a pint of soil taken from a pollution spot 15 yards into the cane. Pint samples taken from the areas within a radius of 1½ feet to 2 yards from this yielded from 0 to 53 hookworm larvae per sample. The environment here was seemingly in every way favorable for migration, yet during the entire time of the investigation, the larvae were always found at more or less definitely localized spots, at the places of soil pollution. That there occurs little or no migration of the hookworm larvae even from centers of very heavy soil infestation has also been shown by Cort and Payne (1922*b*, p. 159) under conditions existing in a cacao grove. The evidence from these field studies and the data from the laboratory experiments given above show that it will be necessary to revise our present conception of the extent of the migrations of infective hookworm larvae, and make it seem very probable that it is only by mechanical means that hookworm larvae can spread from the place of their development.

WHEN PLACES OF SOIL INFESTATION BECOME DRY, DO HOOKWORM LARVAE MIGRATE TO MORE FAVORABLE SITUATIONS?

To test this point, a large number of hookworm larvae were placed on a small spot in a 13-inch dripping pan, containing moist sand 1 cm. in depth. A second area, to which no larvae were added, was located 8 inches away from the first. The whole surface was then evenly sprinkled with water. Water was afterwards added daily to the second area which alone was kept constantly moistened. Ten days later all the sand appeared to be thoroughly dry except that in and around the watered area. This was transferred to a unit of the small isolating apparatus, and upon examination, proved to be negative for hookworm larvae. A small amount of soil taken directly from the original infestation spot was placed on a glass slide, a few drops of water were added, and upon microscopial examination three shrivelled, completely disorganized hookworm larvae were found. The remaining soil of this area was then examined, but yielded no larvae. This experiment indicates that infective hookworm larvae cannot migrate from a drying area to a moist area nearby, but, of course, needs confirmation by a larger series of experiments.

EXPERIMENTS ON PASSIVE MIGRATION.

In two experiments made in the same manner as those summarized in Table 1, except that water was added until the surface of the soils was covered, a fairly uniform distribution of the larvae was found in all zones. In the first, with clay loam soil, where 500 hookworm larvae were added to the infestation center, 72 larvae were recovered from that area, 70 from the second, 17 from the third and 3 from the fourth, eight days after the experiment was started. In the second, with a sand and clay soil, 1,300 larvae were placed in the center of infestation. Eight days later 83 larvae were recovered from Zone 1, 271 from Zone 2, 245 from Zone 3 and 258 from Zone 4.

As these two above experiments differed from those of Table 1 only in the amount of water added, it seemed that in these cases the larvae might have been mechanically carried out of the infestation centers by the surface water. In fact, in the examination of preparations in the counting of larvae, they were often found on the surface film.

To determine the numbers of larvae that might be carried from a given center by this means, the following experiments (see Table 2) were made. Two hundred and twenty-two active hookworm larvae were placed in the center of a 6-inch pudding pan, containing one inch of clay loam soil. The larvae remained undisturbed for several minutes when water was added, until the soil was well covered. The standing water was immediately drawn off with a pipette from all parts of the surface, except that in which the larvae had been placed. This was centrifugated and upon examination of the residue 52 hookworm larvae were found. This showed that 23.4 per cent. of the total number of larvae, placed in the infestation center, had been

TABLE 2.

Showing number of hookworm larvae carried in surface film.

Exp. No.	No. larvae used.	Position of larvae in soil.	No. larvae recovered.	Per cent. recovery.
1	222	No larvae visible..................	52	23.4
2	234	No larvae visible..................	61	26.1
3	100	No larvae visible..................	28	28.0
4	268	Larvae protruding from surface.......	117	43.6
5	127	Larvae protruding from surface.......	38	29.9
6	156	Larvae protruding from surface.......	57	36.5

carried out by the water. Experiments 2 and 3 of this table were made under the same conditions as the first, and their results further illustrate the rôle water plays in the dissemination of hookworm larvae.

In the first three experiments of Table 2 no larvae were visible on the surface of the soil, at the time the water was added. In experiments 4, 5 and 6 of the same table, the larvae had been placed in the soil 15 hours before the water was added. During that time they had established themselves on the surface of the soil, and were found protruding from the prominent soil particles within the infested area. Water was added as before, until the soil was well covered, and upon examination of the residue from the free water slightly higher percentages of the larvae were recovered than in the first three experiments (see Table 2). Apparently when the larvae are well established on the surface of the soil, they are more easily transferred to the surface film.

That hookworm larvae are carried away from centers of soil infestation by the washing of rains, under natural conditions, is indicated by the findings of Cort and Payne (1922, p. 141). Soil samples examined from the drains in the heavily infested area of the cane field studied, showed that the larvae were fairly evenly distributed for a distance of 50 yards. Soil pollution was found only in the higher parts of the drains and their environs. The authors consider that this distribution in the drains is due to the larvae being washed down from the upper ends of the drains, as well as from the areas drained.

To determine the number of hookworm larvae carried on the feet of man, after passage through areas of heavy soil infestation, the soil was scraped from the shoes of three persons, after they had completed a pollution survey in a cane field (see Cort and Payne, 1922*a*, p. 115). Soil obtained in this way was examined for hookworm larvae by the routine method, on six different occasions. The findings varied from 2 to 344 hookworm larvae per person. No appreciable difference was noted in the number of larvae obtained, whether rain had fallen twenty-four hours previous to the survey or not. This is explained by the fact that although the amount of rainfall varied, the soil in the cane was always moist enough to cling to the shoes in considerable amounts. The finding of 344 hookworm larvae in the soil adhering to the shoes of one person is significant. This person had undoubtedly stepped in a "nest" of larvae which was immediately transferred to his shoe. Had he been barefoot and the mud remained on the skin, the resulting infestation would probably have been heavy from this one exposure. Again, had the soil been washed off or dropped on a frequented path, there would have been danger to others.

Domestic animals passing through infested areas likewise enter as factors in the dissemination of hookworm larvae. No actual experiments were made to determine this point, but, from observations made, both in cacao groves and cane fields, it seemed evident that a considerable amount of infested soil may be scattered about in this fashion.

The results of my experiments, as well as field observations, have shown that the hookworm larvae are not migratory during their free life in the soil of Trinidad, but that they can be spread to some extent mechanically. Further investigations are needed to test out, under a variety of conditions, the points brought out in this study.

SUMMARY.

1. Experiments carried on in Trinidad, British West Indies, from May to September, 1922, showed that infective hookworm larvae placed on moist soils do not migrate in periods from 15 hours to 42 days.
2. Experiments show that hookworm larvae may be carried out from centers of soil infestation by surface water, and that they can establish themselves in the new locations when the water recedes.
3. Hookworm larvae were not found to migrate to favorable situations when their original environments become unfavorable.
4. Soil scraped from the shoes of men passing through infested areas was found to contain hookworm larvae. It is possible that paths leading to such areas, as well as the immediate surroundings of dwellings, become centers of infestation by the dropping of such soil.
5. During the time the larvae remained in the soil there occurred a reduction in their numbers, which increased with the number of days the experiment lasted.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. IX.

On the Position of the Infective Hookworm Larvae in the Soil[*]

By DONALD L. AUGUSTINE, B.S.

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While it is known that the soil is the natural habitat of the infective hookworm larvae, only a few observations have been made as to their activities and position in it. Baermann (1917, p. 622), in his studies of the conditions of development and existence of hookworm larvae in the soil, found them to be rather evenly distributed throughout the soil to a depth of 24 cm. He did not find them directly on the surface but close to it. In other experiments, where larvae were studied in soil overgrown with vegetation, he found hookworm larvae constantly in the soil, but never on the plants themselves. No information is given on the exact conditions existing at the time his observations were made. The data from a series of experiments performed by Dr. James E. Ackert, to study the conditions under which hookworm eggs develop and hatch, show that by far the greater number of the hookworm larvae which developed were in the upper half inch of the soil. The complete discussion of these experiments will appear in a later number of this series. Looss (1905, English translation given in Looss, 1911, pp. 421–422) observed mature Ankylostoma larvae on the surface of charcoal cultures when under a high temperature and in a moist atmosphere. He found formations resembling short fungal hyphae, welded together, which upon examination proved to be hookworm larvae. He further observed that the larvae were not present on the surface when it became drier, but upon addition of water would reappear in 20 to 30 seconds. Smith (1905, p. 192) records the same position of the hookworm larvae on the surface of feces, when exposed to the air for a few days under favorable conditions of moisture. The

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[*] This paper is the ninth of the series on the hookworm investigations of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

Georgia State Board of Health (1910) makes the statement that during a rain, or when dew drops collect, hookworm larvae find their way into the water where they remain, if the water does not dry up, until they have an opportunity to penetrate the human body. And when the dew drops dry or the pool of water evaporates the larvae burrow into the moist earth, there remaining until a fresh downpour of rain brings them to the surface again. Dock and Bass (1910) in their book on hookworm disease say that the larvae may crawl up the leaves, stalks or fruit of vegetables. No statement is made of exact observations of larvae doing this. Schultz (1912) found that in cultures from which blades of grass, small sticks, or other objects project, upon which a film of water condenses, hookworm larvae leave the cultures, follow the water film and ascend to the most advantageous point for contact with an eventual host.

Early in the experiments performed in Trinidad, British West Indies, on the migration of hookworm larvae in the moist loam soils (Augustine, 1922) I found them on the surface extending from the larger and more prominent soil particles. The greater number appeared singly like the hairs on young plants, while others were massed together in flame-shaped formations. Upon close examination with a hand lens the larvae seemed to be in constant undulating movement which greatly increased when the slightest stimulus was applied. A portion of the soil upon which several larvae were extended was placed on a glass slide and treated with a few drops of water. Upon microscopical examination both sheathed and unsheathed larvae were found which left the soil and moved with great rapidity to the margins of the preparation. The bit of soil was then broken and spread over the slide. In it several cast-off hookworm sheaths were found. The larvae on the surface of the soil readily transferred themselves to most objects that touched them, but not to insects, such as ants, as well as small Arachnids which passed through and about the infested centers.

Larvae were found on the surface of the soil in all my experiments, as long as their numbers were sufficiently large for observation and the soil was kept moist. In an experiment to test the effect of direct sunlight on the migrations of hookworm larvae (Augustine, 1922, p. 165) they were found extended from the soil particles as long as the soil remained moist, even at a temperature of 101° F. and in direct sunlight. As the surface of the soil became drier they left their more prominent positions and were found in the deeper cracks and crevices, disappearing altogether if the drying continued. If water was then added the hookworm larvae reappeared in a few seconds in their previous positions. This process was repeated several times a day with the same result, until their numbers became so reduced that the reactions could not be observed. It seems clear from this exeriment that sunlight in itself has no effect upon the movements of the larvae, but influences them indirectly by raising the temperature and increasing the rate of evaporation.

In two experiments on the migration of hookworm larvae (Augustine, 1922, p. 168), where water covered the soil, hookworm larvae appeared extended from short sticks, pebbles and larger soil particles which protruded above the water. They were usually motionless, but the application of a slight stimulus, such as blowing over the surface or jarring the pan, would start them to intense activity, especially where they had formed themselves into petal-like clumps. Toothpicks were placed upright in the soil in the infestation centers. A few hours later the larvae were found one third of the distance up the toothpick, but only as far as the moisture had ascended.

During the study of the active migrations of hookworm larvae in grass-covered soil (Augustine, 1922 p. 165) observations were made to discover whether the larvae would climb upon the blades of grass and collect in drops of water, as has been suggested might happen in morning dew. With a hand lens larvae were found at the bases of the stems and on the soil within the centers of infestation. They were also found along the outer dead sheaths of the grass which were moist, but never on the green stalks and blades. The green stalks and blades were cut from and surrounding the infestation centers and washed well in water. This water was then centrifugated and the residue examined with the microscope. No hookworm larvae were found. This test was repeated several times with constantly negative results. The probable reason for the larvae not creeping up the green stalks and into drops of water collected in the axiles of the leaves is that there was no continuous film of moisture present as on the dead stalks and sticks. Their movements seem to be definitely limited by the extent of the moisture film.

In connection with a study carried on by Cort and Payne (1922, p. 149) to determine the sources of infestation with hookworm on a cacao estate an attempt was made to duplicate in the laboratory the conditions existing in this environment, to determine the activities of the infective hookworm larvae under such conditions. In a particular grove of this estate the subsoil was a fine, compact, yellow clay. The surface soil contained an abundance of humus and was of a loose texture and dark. Scattered over much of the surface was a layer of decaying twigs and cacao leaves which during the rainy season were constantly moistened by intermittent showers. Here and there the surface of the yellow clay was exposed, and in other places the humus was free from the leaves and twigs. Soil pollution and infestation was found on all three types of surfaces. The soils, twigs and leaves taken for the laboratory experiment were selected from places widely separated from polluted areas. The yellow subsoil was first placed to a depth of 1¼ inches in a circular pan, 3 feet in diameter. Two thirds of its surface was then covered with the humus, and half of the humus area was carpeted with the leaves and twigs. At the center of each of these surfaces were placed approximately 1000 hookworm larvae, their positions being marked by circles of cord. On the following day a careful examination was made with a hand lens. Numerous larvae were found extended from the soil particles within the infestation centers on the yellow clay and humus soils. The larvae which had been placed upon the leaves were not confined within the center, but were found distributed within a radius of eight inches. They were found on the center margins and prominent veins of the leaves and on decaying bark of twigs, especially where the bark was broken and distinct edges were formed. It is probable that the greater extent of distribution of the larvae on the leaves was not due entirely to their active migration, but was aided by the washing of the water during the process of moistening; for while the water which was added was immediately absorbed by the two soils, it ran off the leaves, from one to another. Larvae were found within the infestation centers of the two soil surfaces at all times until their numbers became greatly depleted. The leaves lost their moisture quickly, and when dry the larvae disappeared to the deeper moisture places, returning to their former position at the next watering.

The position of the hookworm larvae, as found in these and other experiments, is significant since it makes easy the transfer of the larvae from the soil and other infested objects to the human foot.

SUMMARY.

1. Infective hookworm larvae under optimum conditions of moisture and temperature were found to remain on and within the upper surface of the soil.

2. They creep up pieces of wood, decaying vegetation and other objects only as far as the film of moisture extends.

3. Hookworm larvae were not found within the drops of water collected in the axile of the leaves of green plants nor upon the leaves themselves.

4. At centers of soil infestation, where the surface is covered with leaves or twigs, the infective hookworm larvae were found extended from the leaves or twigs when moist, but in the soil beneath when dry.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. X.

EXPERIMENTS ON THE LENGTH OF LIFE OF INFECTIVE HOOKWORM LARVAE IN SOILS.*

BY DONALD L. AUGUSTINE, B.S.

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HISTORICAL ACCOUNT.

The conception of the length of life of the infective hookworm larvae in the soil which is widely accepted can be illustrated by a quotation from Nicoll (1917, p. 163): "These larvae (hookworm), when they reach this final stage have ceased to feed, and they can remain alive for months and even years under suitable conditions, i.e., where there is a sufficiency of moisture and not too great heat. In the laboratory they have been kept alive for over 18 months in plain water, at a temperature of about 60° F. It can hardly be doubted that they will live fully as long under natural conditions, unless it be that they are attacked and devoured by other animals, such as aquatic insects." The evidence which points to an extended period of life for the mature hookworm larvae comes chiefly from the work of the first seven investigators listed in Table 1. These observations (see Table 1) were made upon sheathed larvae, kept in moist feces or water under laboratory conditions in the temperate zone. Further, the inference has often been drawn, as in the above quotation, that if the larvae can remain alive for such periods of time under unnatural surroundings, their span of life would be as great or even greater, when in their natural environment.

The work of the last three investigators summarized in Table 1 needs further discussion, since their observations were made under natural conditions. Leichtenstern (1887, p. 669) found the larvae of *Ancylostoma duodenale* alive in the fecal mass from a brick-yard latrine three months after the laborers had left the field. Stiles and Gardner (1910) found two motionless, but well-preserved sheathed hookworm larvae in sand 151 days after the sample had been taken from under a surface privy. In experiments by these investigators,

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* This paper is the tenth of the series on the hookworm investigations by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

TABLE 1.

Showing results of previous authors' observations on length of life of hookworm larvae.

Authors.	Medium.	Environmental conditions.	Duration of life.
Leichtenstern, 1887	Water Moist feces	Laboratory temperature in Germany	7 months
Galli-Valerio, 1905	Moist feces	Laboratory temperature in Switzerland	13 months
Boycott, 1905.....	Moist feces	Laboratory temperature in London, England	More than 12 months
Loebker and Bruns, 1906...........	Water	By laboratory window, Germany	12 months
Oliver, 1910......	Water	Laboratory temperature, England	15 months
Perroncito, 1913 ..	Water	Laboratory temperature, Italy	8–10 months
Nicoll, 1917......	Water	60° F.	18 months
Leichtenstern, 1887	Fecal material	Abandoned latrine in brickyard, Germany.	3 months
Stiles and Gardner, 1910	Sand	Unheated laboratory during early fall, Wilmington, N. C.	A few alive up 4 months
Baermann, 1917...	Soil covered with sod	In the open shade, Sumatra	6 months

where fecal material containing hookworm eggs was covered with sterilized sand, examinations made four and five months later disclosed no hookworm larvae. These experiments were carried on at unheated room temperature in the early fall at the United States Marine Hospital Station, Wilmington, North Carolina. Baermann (1917, p. 622) in his studies on the conditions of development and existence of hookworm larvae in the tropical climate of Sumatra found that the length of life varied under different conditions of moisture and shade. In one box, where a stool containing hookworm eggs was covered with earth by a sexton beetle and exposed to sun and rain without additional moistening, some slight development took place, but the larvae were found to have died after three weeks. On shaded, sprinkled soil the larvae developed in large numbers, lived for 3 months and then died. In boxes planted with grass extensive development of larvae took place in both sun and shade. He found numerous larvae in the unshaded soil up to three months, and a few up to six months in the shaded. The results of these experiments on the length of life of hookworm larvae in the soil, with conditions as nearly natural as possible, gave a shorter period of life than the others in which the conditions were more unnatural, and suggest that soil does not remain infective as long as has been generally believed.

Leuckart (1868, p. 436), many years ago, pointed out that the mature larvae of *Ancylostoma caninum* (*Dochmius trignocephalus*) live at the expense of reserve food stored in the cells of the intestine during development, and as time passes these cells gradually loose their granules, until the worms become transparent. Looss (1911, p. 419) concluded that if the granules deposited in the body are reserve food stores, which are gradually reabsorbed during the later life of the larvae, a limit is set to the length of life, and death will come when this supply is exhausted. He believed that the length of life would depend on whether the supply of food was consumed slowly or rapidly, and since temperature influences to a high degree the motility of the mature larvae, they would remain alive longer at a low than a high temperature. If we accept this view we should expect the life of the larvae to be the shortest under those conditions of temperature which are most favorable to intense activity, as in the tropics. The results of my experiments carried on in Trinidad, British West Indies, and the field studies of Cort and Payne (1922*a* and *b*) support this view.

MATERIAL AND METHODS.

The hookworm larvae used in the experiments which will be described in this paper were taken from cultures five to eight days old, with the exception of those used in the series on the length of life of unsheathed larvae which were from an eighteen-day-old culture. The same technique was employed in counting and in preparing the soils as in the migration experiments previously described (Augustine, 1922*a*). Two sizes of containers were used, viz., (1) tin pudding pans, six inches in diameter and two inches in depth, and (2) three-ounce glass jars.

DATA ON LENGTH OF LIFE FROM MIGRATION EXPERIMENTS.

Early in my work on the migration of hookworm larvae in soils (Augustine, 1922*a*) I was struck by the great reduction in the numbers of larvae recovered from experiments which had run a few weeks. Table 2 gives the data on the reduction of hookworm larvae in ten of these experiments. Although the experiments were made under favorable conditions, after a period of twenty-one days, there occurred a reduction in the numbers of larvae of over 90 per cent. Stimulated by these findings, experiments were planned to further test this point.

TABLE 2.

Showing reduction in the numbers of hookworm larvae in the migration experiments.

No. Exp.	No. larvae placed in soil.	Type of soil.	Time larvae remained in soil.*	Larvae recovered.		
				Un-sheathed.	Sheathed.	Total.
1	1,000	Moist clay loam	7 days	141	180	321
2	498	Moist sand	10 days	81	10	91
3	512	Moist clay loam	11 days	234	3	237
4	1,100	Moist clay loam	21 days	11	7	18
5	392	Moist clay loam sod	21 days	1	4	5
6	500	Moist red clay loam	22 days	6	3	9
7	361	Moist clay loam sod	30 days	1	1	2
8	500	Moist clay loam	35 days	2	3	5
9	426	Moist clay loam	37 days	0	1	1
10	650	Moist clay loam	42 days	0	1	1

EXPERIMENTS ON LENGTH OF LIFE OF MATURE HOOKWORM LARVAE IN SOILS.

Table 3 gives data from a series of thirty experiments to determine the amount of mass reduction in a known number of mature hookworm larvae in three types of soil, viz., (1) clay loam, (2) sand, and (3) a clay loam soil, under favorable conditions of temperature and moisture. In these experiments the soil was placed to a depth of 1½ inches in thirty of the six-inch pans, ten pans with each type of soil. Three hundred active, sheathed hookworm larvae from cultures five and seven days old were added to each pan. The pans were then placed in a shady place and the soil kept moist throughout the experiment. Examinations, by the isolation apparatus, of the soil from one pan of each type were made weekly for seven weeks and the larvae recovered counted. As the seventh examination gave negative findings for hookworm larvae the eighth examination was made three days later. As this also proved to be negative for all three soils the experiment was closed. The results of these experiments (Table 3) indicate a rapid dying off of the larvae within the first two weeks of the experiment. If the time of development in the cultures is added, it is seen that in three weeks after the eggs left the host 90 per cent. of the larvae which developed to the infective stage had perished. The significant points brought out in this series (Table 3) are (1) the high reduction occurring within two or three weeks, (2) the fairly constant number of larvae remaining alive up to seven weeks, and (3) a complete dying

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* To give the total age of the larvae, at the conclusion of the experiment, the time of their development in the cultures (5 to 7 days) must be added to the time they remained in the soil.

TABLE 3.

Showing mass reduction of hookworm larvae in 3 types of soil.

In each of the experiments 300 sheathed hookworm larvae, taken from cultures 5 to 7 days old, were placed in 6-inch pudding pans containing 1½ inches of soil. Experiments started July 5, 1921.

Exp. No.	Date of examination.	Clay loam soil.				Sandy soil.				Clay loam soil.			
		Larvae recovered.		Total.	Per cent. reduction.	Larvae recovered.		Total.	Per cent. reduction.	Larvae recovered.		Total.	Per cent. reduction.
		Unsheathed.	Sheathed.			Unsheathed.	Sheathed.			Unsheathed.	Sheathed.		
1	July 12	52	4	56	81.3	123	9	132	56	30	9	39	87
2	July 19	16	1	17	94.3	38	1	39	87	10	2	12	96
3	July 26	14	5	19	93.6	13	0	13	95.6	15	3	18	94
4	August 2	13	5	18	94	11	1	12	96	0	0	0	100
5	August 9	5	0	5	98.3	6	8	14	95.3	6	8	14	95.3
6	August 16	1	14	15	95	4	0	4	98.6	2	7	9	97
7	August 23	0	0	0	100	0	0	0	100	0	0	0	100
8	August 25	0	0	0	100	0	0	0	100	0	0	0	100
9 and 10	Not examined												

out after that time. It would therefore appear that the activities of the larvae are at first intense, which is followed almost immediately by a high death rate.

TABLE 4.

Showing mass reduction of hookworm larvae in a clay loam and sandy soil.

In all these experiments the 3-oz. glass jars were used, filled ¾ full with soil.

Exp. No.	All experiments started July 19.	Clay loam soil.					Sand.				
	Date of examination.	Number of larvae placed in soil.	Larvae recovered.		Total.	Per cent. reduction.	Number of larvae placed in soil.	Larvae recovered.		Total.	Per cent. reduction.
			Unsheathed.	Sheathed.				Unsheathed.	Sheathed.		
1	July 22	300	128	161	289	3.6	300	83	159	242	19.3
2	July 25	300	115	144	259	13.6	311	99	96	195	37.2
3	July 28	300	69	54	123	59.0	321	90	67	157	51.0
4	July 31	300	42	29	71	76.3	308	107	70	177	42.5
5	Aug. 3	300	59	29	88	70.6	303	29	9	38	87.4
6	Aug. 6	300	33	12	45	85.0	316	48	3	51	83.8
7	Aug. 9	300	40	7	47	84.3	304	40	15	55	81.9
8	Aug. 12	300	5	0	5	98.3	331	38	0	38	88.8
9	Aug. 15	300	13	5	18	94.0	325	26	1	27	91.6
10	Aug. 18	300	3	0	3	99.0	304	21	1	22	92.1

Table 4 gives the results of a series of experiments similar to those summarized in Table 3, except that the examinations were made every third day and the containers were the three-ounce glass jars. As the data show, the greatest reduction occurred about two weeks after the experiment was started, or about three weeks after the deposition of the eggs.

THE RELATION OF LOSS OF SHEATH TO LENGTH OF LIFE.

As has been previously stated (Cort, Augustine, et al., 1922) a large proportion of the mature hookworm larvae lose their sheaths in the soil. This discovery immediately introduces a new factor into the study of the length of life of the infective hookworm larvae. Three questions present themselves, viz., (1) Does the loss of the protective sheath directly shorten the life of the larvae? (2) Does the loss of sheath make the larvae more susceptible to unfavorable conditions of environment and thus shorten their life under certain conditions? and (3) Does the loss of sheath render the larvae non-infective, which would be the equivalent of terminating their lives?

TABLE 5.

On the mass reduction of hookworm larvae in loam soil from cane field.

Ten experiments started July 18, with 100 unsheathed larvae in each. All larvae were obtained from same culture, 18 days old.

Experiment number.	Date of examination.	Number of larvae recovered.	Per cent. reduction.
1	July 21	87	13
2	July 24	52	48
3	July 27	51	49
4	July 30	15	85
5	August 2	13	87
6	August 5	4	96
7	August 8	7	93
8	August 11	0	..
9	August 12	0	..
10	August 12	0	..

Table 5 gives the data on a series of experiments on the length of life of unsheathed hookworm larvae in clay loam soil under favorable conditions. The larvae used in these experiments were isolated from soil on which a stool containing hookworm eggs had been placed eighteen days before. Of the 1,300 larvae obtained only 10 were found enclosed in sheaths. The experiments were made under the same environment as those with the sheathed forms. Examinations were made every third day as long as the results were positive. After the first negative appeared, which was 24 days after the experiments were started, or 42 days after the stool had been placed on the soil, the remaining jars were examined on the day following. These were likewise negative. While this series of experiments indicates that the life of the unsheathed hookworm larvae may be similar to that of the sheathed forms it must be taken into consideration that there undoubtedly had occurred a great reduction in the numbers of larvae before isolating them from the culture, and that the larvae used in the experiment were the survivors of this reduction.

While no experiments were made to test the comparative resistance of the unsheathed and the sheathed larvae, it was found that upon the addition of a few drops of dilute formalin (See Cort, Ackert, et al., 1922, p. 5) to the preparation before counting, the unsheathed larvae quickly succumbed, while the sheathed forms were stimulated to greater activity, and could resist the formalin for a considerable time. This observation indicates, as would be naturally inferred, that unsheathed larvae are less resistant to unfavorable conditions than sheathed. This would then lead to a more rapid dying of the unsheathed larvae whenever conditions became unfavorable. Since our present knowledge of the resistance of the mature hookworm larvae to various conditions is based entirely upon studies of the sheathed forms it will be necessary to repeat much of this work with the unsheathed larvae, to determine what actually happens under natural conditions.

That sheathed hookworm larvae complete their second ecdysis, while penetrating the skin, and become adults in the intestines of man has been conclusively demonstrated by a number of investigators. Whether the loosing of the sheath in the soil renders the larvae non-infective, as far as man is concerned, which would be equivalent to their death, is not yet known. To gain information on this point unsheathed larvae were applied to the shaven belly of a male white rat, sixteen months old. Upon examination with a binocular microscope the larvae were seen to quickly spread themselves over the surface of the skin as far as the moisture extended. As the moisture around the larvae evaporated penetrating movements were started. The anterior ends of the worms would be lifted into the air and then turned toward the skin again, touching here and there as if seeking some port of entry. In one particular instance a larva was seen to leave the shaven area, to settle at the base of a hair and push one third of its body into the hair follicle. Then after a moment's rest the posterior end swung up parallel with the hair and with several slow twists the worm moved further into the skin and again rested. This movement was repeated several times until the tail of the worm vanished beneath the surface of the skin. Two other unsheathed larvae were seen to penetrate the skin within the shaven area. The entire process took less than a half hour. One hour later water was applied to the infected spot and upon microscopical examination no larvae were found. While this observation only shows that unsheathed hookworm larvae can penetrate mammalian skin, it seems very probable that they can also reach the intestine and grow to maturity. Further experiments are needed to clear up this point.

DISCUSSION OF RESULTS.

This experimental evidence on the length of life of the infective hookworm larvae in the soil is supported by the field observations of Cort and Payne (1922*a*, p. 138). In one instance soil pollution was found near the edge of a cane field and soil samples from this area showed it to be an intense center of infestation. This area was visited frequently at later intervals, but no further evidence of soil pollution was found. Soil samples, taken three weeks later from the same place, yielded but nine hookworm larvae, and later examinations showed a complete dying out of the larvae. More striking still is their study of the mass reduction of hookworm larvae in a chosen area of this field which, before educational work, sanitation, and treatment were started, was found to have a very heavy infestation with hookworm larvae. In less than three weeks after the reduction of soil pollution, which immediately followed the introduction of control measures, a second series of examinations was made. The results of these examinations showed a reduction of over 90 per cent. from the first series. Subsequent examinations of samples taken from this area showed still further reductions. In about six weeks after pollution of the soil had been stopped this once heavily infested strip of cane field had become practically free from hookworm larvae. Similar results were obtained during their studies on the sources of hookworm infestation in a cacao grove (Cort and Payne, 1922*b*). Although soil pollution was not checked samples of soil taken from grossly polluted areas, six weeks after treatment, showed a marked reduction in the number of infective hookworm larvae present.

The results of these laboratory experiments and field observations show that the life of the hookworm larvae in the soils of Trinidad is limited to a few weeks. Whether this finding is constant under a variety of conditions is not known, and experiments are planned to test this point. These results are very different from those obtained with sheathed larvae under laboratory conditions at lower temperatures. It would seem that the differences in these results can be explained by differences in the conditions of the experiments. The conditions of the experiments of the early workers (see Table 1), in which larvae were kept alive for such long periods, were such that the activities of the larvae were greatly reduced and the protective sheaths retained. In my experiments in Trinidad the daily range of temperature during the course of the work was from about 74° F. to 94° F., a temperature which, if other conditions were favorable, tended to keep the larvae active. Further it was found that a large proportion of the larvae lost their sheaths in the soil. While it is not possible from the information at hand to find any direct correlation between the process of unsheathing and the death rate under favorable conditions, it was found that the unsheathed larvae are more susceptible when in unfavorable environments. As the unsheathing of the larvae appears to be a continuous process, as long as the soil remains infective, the newly unsheathed larvae would be constantly killed when becoming exposed to diverse conditions, even though sheathed larvae were able to withstand the diversity. It can be concluded, therefore, that conditions favorable to intense activity and to the loss of the sheath will tend to shorten the life of mature hookworm larvae in the soil.

SUMMARY.

1. From the observations of various investigators on hookworm larvae under unnatural conditions the opinion has become current that they live for months or even years in the soil, under favorable conditions of temperature and moisture.

2. Laboratory experiments carried on in Trinidad, British West Indies, from May to September, 1921, show that a rapid reduction in the number of hookworm larvae occurs in soils of various types, and that the extent of their life is limited to about six weeks.

3. The completion of the second ecdysis of the infective hookworm larvae in the soil presents a new factor in determining their length of life, which was not found to directly shorten the life of the larvae under favorable conditions, but renders them more susceptible when in unfavorable environments.

4. Experimental evidence indicates that the loss of sheath does not render the mature larvae non-infective which would be the equivalent of their death.

5. The conclusion can be drawn that environmental conditions, such as tropical temperatures which tend to increase the activity of the mature hookworm larvae, will shorten their lives by the more rapid using up of the stored food material.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE.

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INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. XI. VERTICAL MIGRATION OF INFECTIVE HOOKWORM LARVAE IN THE SOIL (PRELIMINARY REPORT).*

BY FLORENCE KING PAYNE. M.D.

(Received for publication February 1st, 1922.)

Plates XI to XIII.

INTRODUCTION.

There have been a number of studies of the migration of hookworm larvae on the surface of soil and on the walls of mine shafts, but investigations of their travels beneath the surface have been hampered by the lack of adequate means of isolating them from surrounding media. The development of a successful method of isolation (Cort, Augustine, Ackert and Payne, 1922; Baermann, 1917) has given an opportunity for the pursuit of studies of such migrations.

The vertical migrations of hookworm larvae may affect practical hookworm control in two chief ways: First, by the possible retirement of the larvae during dry weather from the surface to zones of more moisture. Second, by the possible migration upward of larvae which are beneath the surface. Studies of such migrations should assist in the evaluation of methods of excrement disposal, especially where burial is practiced or pit latrines are used. The present study considered only the second phase of vertical migration. Each experiment was based on the assumption that excrement containing fully developed larvae had been buried at a known distance beneath the surface. The object was to determine whether larvae were likely to reach the surface in sufficient numbers to produce an infective focus.

METHODS.

Samples of the three types of soil which were used were obtained in quantities of fifty pounds to one hundred pounds. Each was thoroughly mixed to obtain uniform distribution of the various constituents. Portions of each sample were dried at a temperature of about 60° C. and were pulverized. It was determined by experiment that drying at this temperature was sufficient to kill any nematodes or larvae, but was not high enough to char organic matter. The dried soil was kept as the stock from which material for the various experiments was obtained.

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* This paper is the eleventh of the series of hookworm investigations made by the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, with the cooperation of the International Health Board, Rockefeller Foundation.

Containers for the experiments were of various sizes. The ones most used were tin sleeves, about three inches in diameter and five inches high. They were slightly tapered so that two or more could be joined to obtain additional length. Other containers of various sizes and forms were used in a few experiments and will be described with them.

Except where otherwise specified, only well developed *Necator americanus* larvae were used. The methods of culture and of isolation of the larvae have been given in an earlier paper of this series (Cort et al., 1922). In nearly all experiments the larvae were counted and were placed in a thin layer of moist soil on the bottom of the container.

The determination of the extent of upward migration accomplished by the larvae was made by removing the soil from the container in layers of measured thickness. Each layer was placed in an isolation apparatus and the larvae were recovered from it according to the method to which reference has been made. The larvae recovered from each layer were counted.

EXPERIMENTAL WORK.

Experiments were conducted to test the effect of the following factors on the upward migration of hookworm larvae:

1. Moisture content.
2. Conditions simulating the rise of ground water.
3. Type of soil.

Another series of experiments was made to study the disappearance of nutritive material from the bodies of the larvae at various stages of migration.

The first experiments were designed to test the effect on vertical migration of different degrees of moisture, uniformly distributed through the soil. In all of these experiments soil from the same lot

TABLE 1.

Influence of moisture content on vertical migration of hookworm larvae.
(The zones are measured from the bottom of the dirt in the containers, and represent the distances above the place of introduction of the larvae.)

Experiment.	Moisture in per cent.	Days.	Larvae inoculated.	Zone 1.	Larvae recovered from Zone 1.	Zone 2.	Larvae recovered from Zone 2.	Zone 3.	Larvae recovered from Zone 3.	Zone 4.	Larvae recovered from Zone 4.	Zone 5.	Larvae recovered from Zone 5.	Zone 6.	Larvae recovered from Zone 6.	Zone 7.	Larvae recovered from Zone 7.
1	41.4	4	507	0–¼″	51	¼″–1″	147	1″ –1¾″	9	1¾″–2½″	4	2½″–3″	0	....	....	....	....
2	41.4	9	611	0–¼″	30	¼″–1″	7	1″ –1¾″	2	1¾″–2½″	2	2½″–3¼″	0	3¼″–4″	0	....	....
3	40.0	6	455	0–½″	14	½″–1″	0	1″ –1½″	0	1½″–2″	0	2″ –2½″	0	2½″–3½″	0	....	....
4	42.2	4	4400	0–½″	962	½″–1″	83	1″ –1½″	25	1½″–2″	9	2″ –2½″	0	2½″–3″	0	3″–4″	0
5	40.6	13	995	0–½″	0	½″–1″	0	1″ –1½″	0	1½″–2″	0	2″ –2½″	0	2½″–3⅛″	0	....	....
6	42.7	7	4530	0–½″	345	½″–1″	51	1″ –1½″	1	1½″–2″	12	2″ –2½″	3	2½″–3″	4	3″–3½″	2
7	40.6	6	560	0–½″	91	½″–1″	17	1″ –1½″	0	1½″–2″	0	2″ –2⅜″	6	....	....	....	....
8	Water-logged	7	1137	0–½″	2	½″–1″	0	1″ –1½″	0	1½″–2″	0	2″ –2½″	0	2½″–3⅝″	0	....	....
9		3	347	0–¾″	2	¾″–1½″	2	1½″–2½″	1	2½″–3½″	0	3½″–4½″	0	....	....	....	....
10		2	1500	0–¾″	163	¾″–1½″	5	1½″–2¼″	0	2¼″–3″	0	3″ –3¾″	0	....	....	....	....

was used. It was typical of the soil in the region of Princes' Town and was made up of a mixture of marl, clay, and humus. For convenience it is designated in the description of the present experiments as "garden soil." The standard tin sleeve containers were used.

The experiments were performed during the rainy season and specimens of soil obtained from fields which were known to harbor hookworm larvae contained from 33.5 to 48.7 per cent. of water. In the laboratory, however, it was found impracticable to prepare a mixture of dried soil and water with uniform distribution, in which there was less than 40 per cent. of water. In the experiments with water-logged soil the earth was saturated throughout, and additional water was added so that the surface of the sample was always submerged.

Table 1 shows the results of a series of ten experiments which were made to test the influence of the moisture content on vertical migration of hookworm larvae. From specimens of which the moisture content was 40 to 42.7 per cent. (Table 1, Experiments 1–7), 1877 larvae were recovered. Of these, 1798 or 95.7 per cent. were in the lowest inch of the specimen; that is, in the periods of four to thirteen days between inoculation and recovery there was no evidence of effective migration. From the specimen left for nine days, only 6 per cent. of the larvae originally used were recovered, and from the specimen left for thirteen days no larvae were recovered.

From specimens which were water-logged (Table 1, Experiments 8–10), 175 larvae were recovered. Of these, only 8 had migrated more than three fourths of an inch. The supernatant water on these specimens was examined daily for larvae, and none was found in any experiment. It would appear that the death rate in these specimens was high.

The failure of extensive vertical migration to occur under conditions of relatively uniform distribution of moisture led to the attempt to simulate some conditions existing in nature. Various efforts were made to bring about experimental conditions which would resemble those existing in the soil during the rise of ground water from the water table to the drying surface.

In experiment No. 11 (see Table 2), dry soil was placed in a glass tube three eighths of an inch in diameter to a depth of three and one half inches. More than one thousand larvae were placed in the lower end on a little moist earth. This end was closed with a stopper into which was inserted a very slender glass tube bent in the form of a U.

The tube of earth was then placed in a vertical position. Water was introduced, a few drops at a time, through the U tube. Care was taken that the level of the water in the U tube should not be higher than the lower end of the column of earth in the larger tube. In the course of a few hours water had permeated throughout the soil. Of 345 larvae which were found in the top one fourth of an inch of the earth on the following day, 117 were recovered from the uppermost lump of earth which measured about one eighth of an inch in diameter.

In experiment No. 12 (see Table 2), one of the standard tin sleeves was used. This was set on a flat tin surface, and the larvae were placed in the bottom in a bit of moist earth. Dry earth was then poured into the sleeve to a depth of four inches, and in the center additional earth was piled to form a cone one half inch in height. A little water was placed on the flat surface outside the container, and it was renewed as rapidly as it was taken up by the soil. The surface of the soil in this container was examined from day to day. After twenty-four hours it was estimated that about 300 larvae could be found with a binocular microscope. The container was kept open and no attempt was made to prevent drying of the surface. After a few days only empty sheaths were visible on the surface of the higher lumps of earth, while larvae could be seen at lower levels. A control experiment was conducted in the same manner, but without larvae, and on the eighth day the moisture content of the soil was found to be 42.3 per cent.

In order to eliminate the possibility of larvae ascending the walls of the container instead of migrating through the earth, experiment No. 13 (see Table 2) was performed, with a container five inches square. This experiment was carried out in the same manner as No. 12, but drying for such a long time was not permitted.

In experiment No. 14 (see Table 2) there was used a deeper layer of earth than in the previous experiments, and in order to obtain a continuous water column from the bottom to the top, it was necessary to moisten the top layers of earth.

In the four experiments recorded in Table 2 migration was more successful. Of 1094 larvae recovered, only 165 or 15.1 per cent. were found within one inch of the bottom of the container. 814 or 74.4 per cent. had migrated three inches or more before recovery; 235 had migrated four inches or more, and 169 five inches or more. The greatest distance noted was in the case of three larvae, in experiment No. 14,

TABLE 2.

Influence of rise of ground water on vertical migration of hookworm larvae.

Experiment.	Days.	Larvae inoculated.	Zone 1.	Larvae recov'd from Zone 1.	Zone 2.	Larvae recovered from Zone 2.	Zone 3.	Larvae recovered from Zone 3.	Zone 4.	Larvae recovered from Zone 4.	Zone 5.	Larvae recovered from Zone 5.	Zone 6.	Larvae recovered from Zone 6.	Zone 7.	Larvae recovered from Zone 7.	Zone 8.	Larvae recov'd from Zone 8.
11	1	1000	0–¼"	64	¼"–1"	101	1" –1¾"	36	1¾"–2½"	39	2½"–3¼"	8	3¼"–3½"	345	....	....	....	...
12	8	600	0–1"	0	1" –2"	1	2" –3"	12	3" –3½"	53	3½"–4"	172	4" –4½"	32	....	....	....	...
13	4	?	0–1"	0	1" –2"	2	2" –3"	5	3" –4"	6	4" –5"	37	5" –5½"	105	5½"–6¼"	46	....	...
14	10	1530	0–1½"	0	1½"–2½"	1	2½"–3½"	1	3½"–4½"	3	4½"–5½"	10	5½"–6½"	11	6½"–7½"	1	7½"–8½"	3

which were recovered from zone seven and one half to eight and one half inches from the bottom.

The experiments which have been described involved studies of migration in only one type of soil, and this a type which is common in relatively restricted areas. In order to determine whether migration might be more successful in other soils a preliminary series of experiments was made with two others which were available at the time. One of these was composed almost entirely of very fine white sand, the other was of sand mixed with finely divided particles of red clay. The experiments with soils were conducted in small containers with a depth of soil of about two inches. The technique was identical with that described in the experiments above which simulated the rise of ground water, except that the larvae were not recovered. The success of migration was tested by frequent search for larvae on the surface of the soil by means of a hand lens or binocular. Each experiment was controlled by a similar preparation in which garden soil was used.

Six series of experiments were made in the attempt to establish conditions under which successful migration would occur in these soils. The experiments with the white sand were uniformly unsuccessful. No larvae were found on the surface of this soil. In the case of the mixture of sand and red clay, there were found at the end of twenty-four hours in each experiment a few larvae, but never so many as were seen on the garden soil control.

The results of the experiments which have been described indicate that in the types of soil which have been studied vertical migration takes place through only a limited range, except under the most favorable conditions. Studies of the larvae, used in the experiments, showed that there was little or no migration, if the food granules were sparse, and anatomical details were readily made out. Larvae, which in the beginning were so thickly studded with granules that very little internal structure could be distinguished, showed, after migration of a few inches, a great diminution in granules and a general appearance similar to that pictured by Looss (1911) in a larva reared in a medium with insufficient nutriment. These observations led to a series of experiments to study the diminution in the number of granules in relation to the various distances migrated.

Six different lots of well developed and nourished larvae were used for this experiment, and with each lot several preparations of different depths were made according to the technique described for the experiments simulating the rise of ground water. The distribution of food granules was studied in the larvae of each lot, in the larvae which came to the surface of the preparations, and in the larvae which were recovered from various zones of the deeper preparations. Photomicrographs were made of typical larvae of each lot and of larvae from each stage of migration. The preparation of the photomicrographs was done by Dr. Geo. C. Payne, and acknowledgment is made to him for his assistance as well as to Dr. K. S. Wise, Surgeon-General, and Dr. Baeza, Acting Government Bacteriologist, for the use of necessary apparatus.

The experiments were conducted under fairly uniform temperature conditions. Room temperature varied from about 72 to 87° F. All experiments were carried through in as short a time as was consistent with the height of migration which was desired. Even the highest migration took place within less than a week from the preparation of the lot, and four days from the actual inoculation of the soil. Portions of the supply of stock larvae were retained in each instance and were kept in clean water as controls to show the changes in the distribution of food granules in inactive larvae. Observations on the controls showed that there was no appreciable diminution in the number of food granules during the period of any experiment. In interpreting these experiments it is to be understood that the total distance travelled by a larva is much greater than the vertical distance of apparent migration. Hand lens studies of the activities of larvae in vertical glass tubes containing earth showed that a larva frequently traverses the same zone many times before finally leaving it for a higher one.

Diminution in the number of food granules was shown after migration of one inch, which was the smallest distance at which studies were made, and when only eighteen hours had elapsed from the time of inoculation. The loss of granules was apparently progressive and constant with farther migration and with the passage of more time, so long as activity was maintained. Because of the devious courses taken by the larvae in their travels there was a certain amount of variation in the diminution of granules in larvae from the same lot and the same zone, but when representative individuals were selected from each zone the disappearance of the granules could be followed with a fair degree of accuracy. There appeared to be an early diminution in the number of granules in the anterior portion of the larvae, and this was followed by an apparent increase in the width of the lumen of the intestine, probably due to a decrease in depth of the intestinal cells. The accompanying figures show a characteristic series of such changes in larvae from one lot, and from different zones of migration up to six and three fourths inches (See Plates XI to XIII, Figs. 1–11).

SUMMARY.

1. In garden soil with uniform conditions of moisture throughout the sample very few *N. americanus* larvae migrated more than two inches above the point of inoculation.

2. In water-logged garden soil there was apparently a very high death rate and there was little migration.

3. In garden soil in which the conditions of rising ground water were simulated 74.4 per cent. of the larvae recovered had migrated three inches or more, 15.4 per cent. had migrated five inches or more, while a few had migrated more than seven and one half inches.

4. Migration was apparently more difficult in the specimens of fine sand and the mixture of clay and sand than in the specimens of garden soil.

5. The food supply carried by *N. americanus* larvae apparently restricts the range of their vertical migration to narrow limits, since a distinct reduction in the granules in the intestinal wall could be noted after vertical migrations of only an inch or two, and almost a complete loss of these granules came about in those which had migrated for five or six inches.

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PLATE XI.

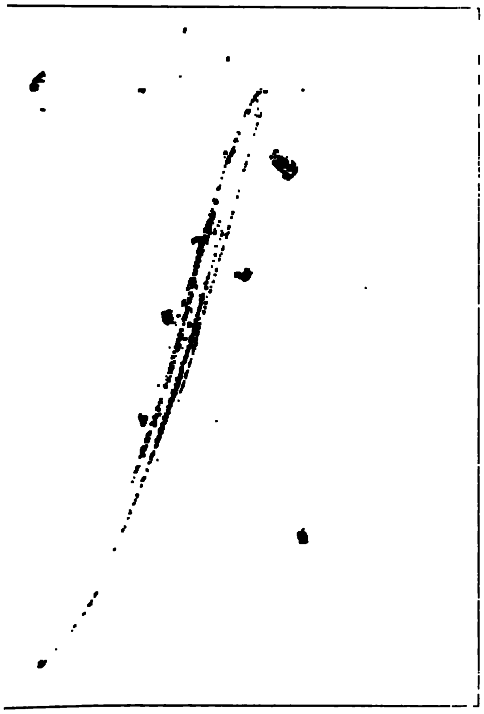

Fig. 1.

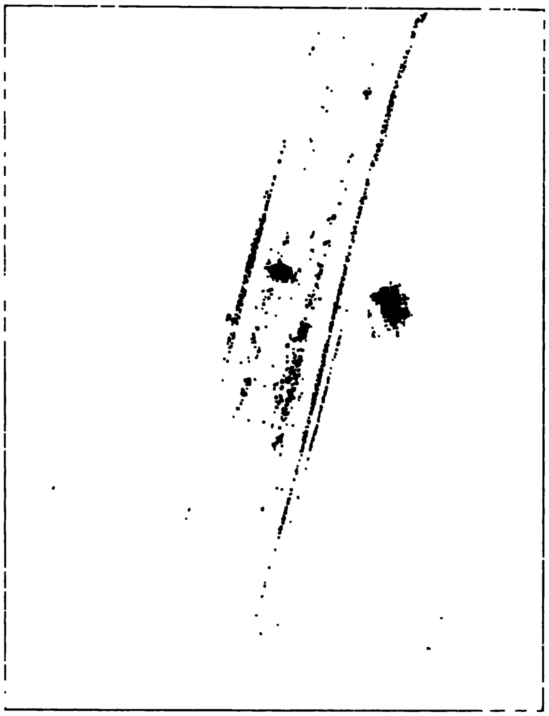

Fig. 2.

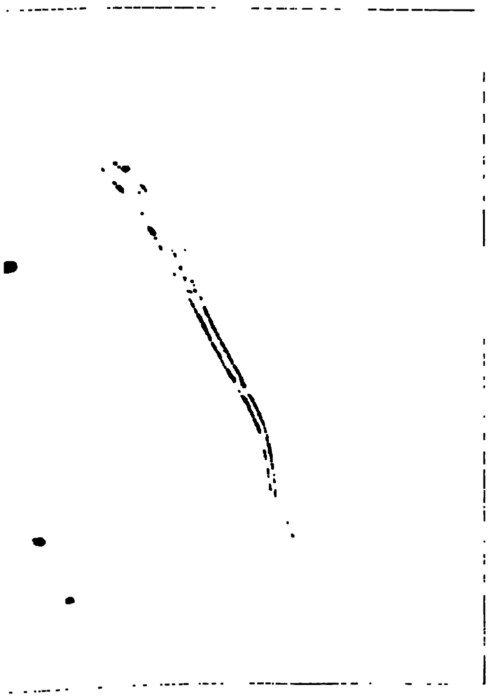

Fig. 3.

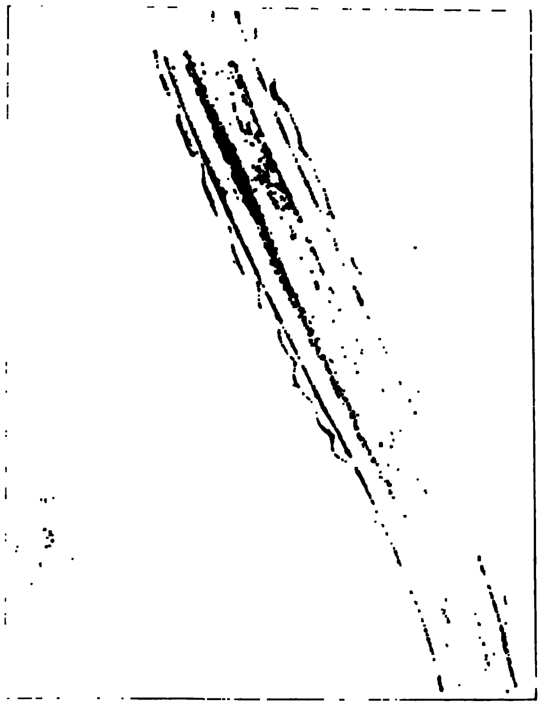

Fig. 4.

PLATE XII.

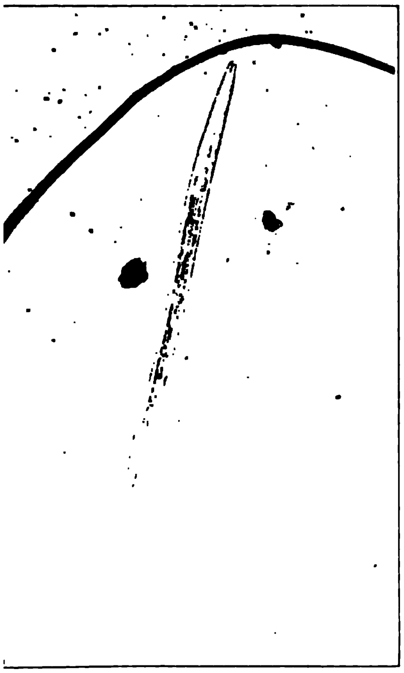

FIG. 5.

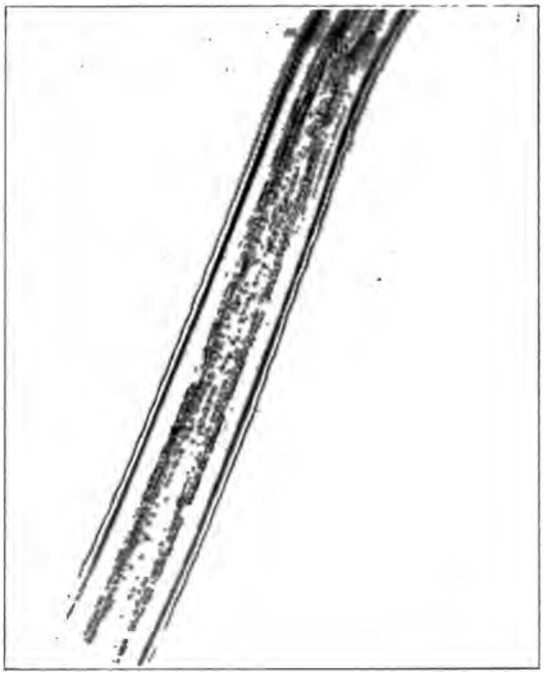

FIG. 6.

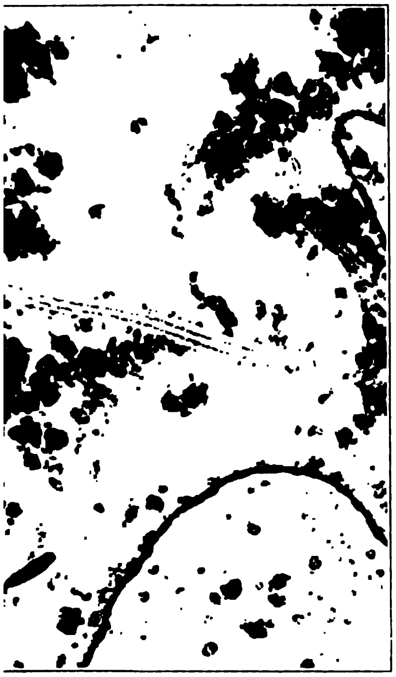

FIG. 7.

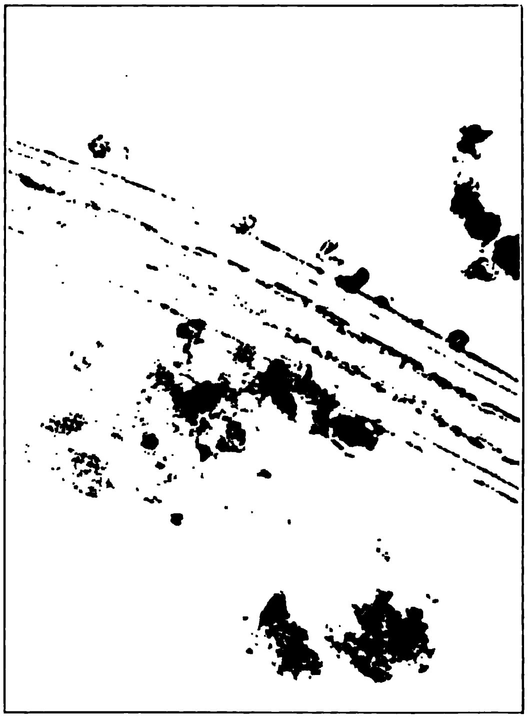

FIG. 8.

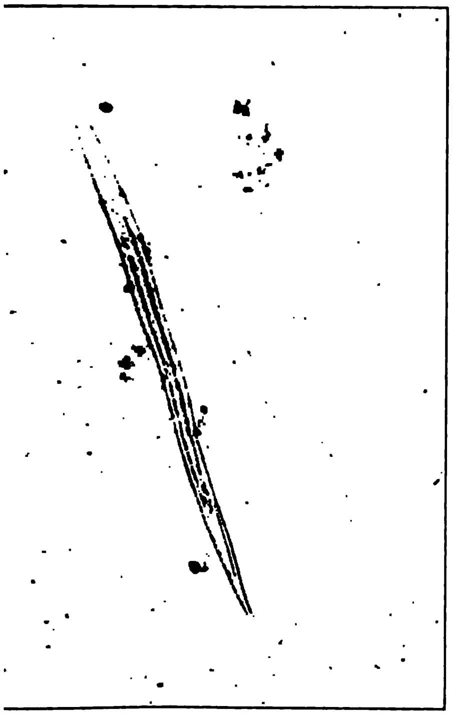

FIG. 9.

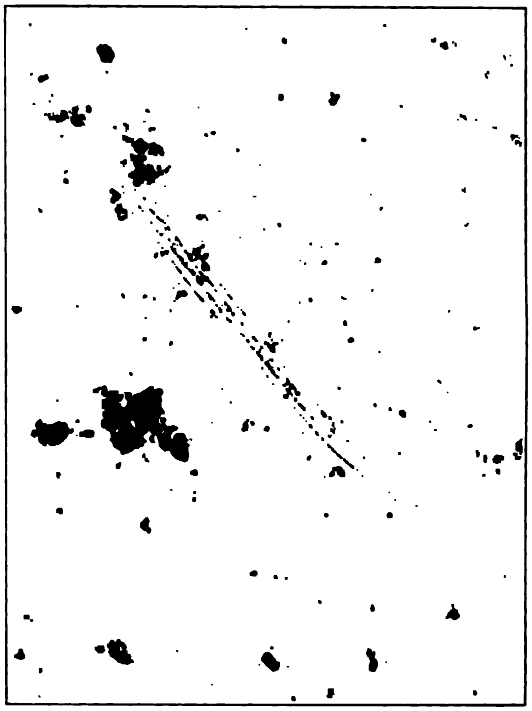

FIG. 10.

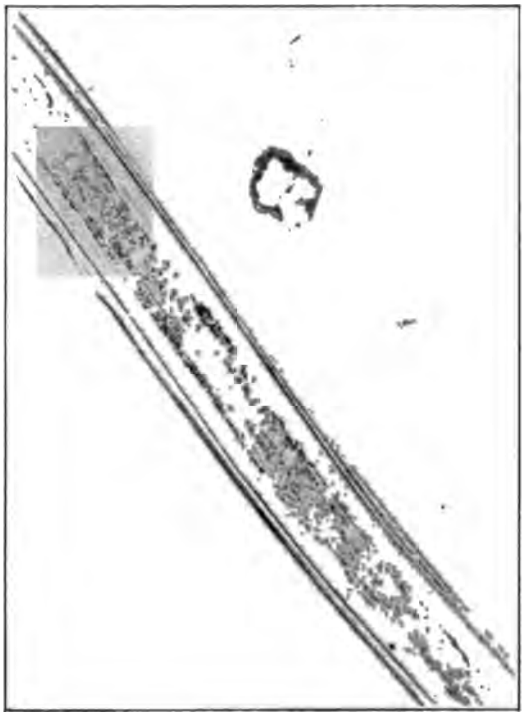

FIG. 11.

EXPLANATION OF PLATES.

Plate XI.

FIG. 1. Larva from lot No. 4, before migration, low-power.

FIG. 2. Larva from lot No. 4, before migration, high-power.

FIG. 3. Larva from lot No. 4, after migrating a vertical distance of two inches, low-power.

FIG. 4. Larva from lot No. 4, after migrating a vertical distance of two inches, high-power.

Plate XII.

FIG. 5. Larva from lot No. 4, after migrating a vertical distance of three and one half inches, low-power.

FIG. 6. Larva from lot No. 4, after migrating a vertical distance of three and one half inches, high-power.

FIG. 7. Larva from lot No. 4, after migrating a vertical distance of four and one half inches, low-power.

FIG. 8. Larva from lot No. 4, after migrating a vertical distance of four and one half inches, high-power.

Plate XIII.

FIG. 9. Larva from lot No. 4, after migrating a vertical distance of six and one half inches, low-power.

FIG. 10. Larva from lot No. 4, after migrating a vertical distance of six and three quarters inches, low-power.

FIG. 11. Larva from lot No. 4, after migrating a vertical distance of six and three quarters inches, high-power.

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VARIATION AND INHERITANCE IN SIZE IN TRYPANOSOMA LEWISI[1]

1. LIFE-CYCLE IN THE RAT AND A STUDY OF SIZE AND VARIATION IN "PURE LINE" INFECTIONS[2]

BY W. H. TALIAFERRO

DEPARTMENT OF MEDICAL ZOOLOGY OF THE SCHOOL OF HYGIENE AND PUBLIC
HEALTH, JOHNS HOPKINS UNIVERSITY

Communicated by R. Pearl, MAY 2, 1921

The flagellate, Trypanosoma lewisi, is a non-pathogenic blood parasite occurring in various species of rats all over the world. It is known to be transmitted from rat to rat by the rat flea. This trypanosome was selected for the present work because it occurs in the latitude of Baltimore and the vertebrate and invertebrate hosts can easily be reared in the laboratory.

The general plan of the present work on size in T. lewisi is to make a careful study of size and variability in a pure line and then with this background to attempt to explain the facts observed in infections occurring in nature. After a pure line infection was obtained the following questions were attacked: (1) What are the mean and the coefficient of variation? (2) Does growing the same "pure line" in different vertebrate hosts cause significant differences in the mean size or in the coefficient of variation? (3) Does passage of the "pure line" through the invertebrate host cause significant differences in the mean or coefficient of variation? This question gives us a chance to test the possibility that passage of the "pure line" through the invertebrate host may cause a splitting up of the "pure line" into heritably diverse lines. After a study is made of these questions we can attack the final one: (4) Does an infection occurring in nature consist of a large number of "pure lines" such as has been described by Jennings[3] and others in free-living protozoa which differ among themselves but are *per se* constant in size? As will be seen later, these questions cannot be approached with any degree of exactness until a thorough study is made of the changes in mean size and variability throughout the course of an infection. The present paper deals with these changes in size and variability and with the first of the questions enumerated above. The other three questions are to be taken up in a later report.

A study of inheritance in a parasitic protozoon such as T. lewisi is of interest from several points of view. In the first place the results are of interest from a comparative standpoint when considered in the light of recent advances in our knowledge of the genetics of free-living species. In the second place, the results may be of value in the interpretation of the results of the many studies on the production of strains of parasitic organisms which exhibit new characteristics. Finally the work is the first of a program of investigations, the ultimate object of which is a study of the mechanism of the formation of new lines exhibiting such characters as arsenic-fastness and the inheritance of these characters after passage through both the vertebrate and the invertebrate hosts.

While lack of space prevents a discussion of technique in detail, it may be noted that every precaution was taken to use microscopical technique such that the trypanosomes would be free from shrinkage and distortion. All measurements were made from camera lucida outlines drawn at a magnification of ×3000. The unit used in measuring the drawings was 3 mm.; consequently all of the determinations given in this paper are in actual microns. In making the determinations 100 specimens, taken at random without selection, were drawn in each case. In isolating single organisms with which to start "pure line" infections, a sensitive mercury pipette was used in conjunction with a Barber pipette holder. Figure 1 is a diagram of a trypanosome indicating the various parts of the organism and the distances measured in this work. The names of the parts of the trypanosome run vertically and the abbreviations of the six distances run horizontally.

Size and Variation throughout a "pure line" Infection.—The infection in the rat can be divided into three periods: (1) the incubation period lasts from 1–7 days and is the time which elapses between inoculation and the first appearance of the trypanosomes in the blood. (2) The multiplication period starts with the first appearance of the trypanosomes in the blood and lasts for 10–25 days. This period is characterized by the great variations in size due to the growth and the multiplication of the trypanosomes. (3) The period of "adult" infection follows the second period and is characterized by the absence of all growth and multiplication. This period lasts from one to many weeks at the end of which time the trypanosomes disappear from the blood and the rat is immune to another infection with T. lewisi. It is apparent that in an organism which shows a cycle of growth and division such as characterizes T. lewisi, we must make all comparisons of size and variability at the same stage in the cycle, and that if there is a period in which there is no growth and division comparisons should be made during this period.

A study of the changes in the means and in the coefficients of variation of the different parts of the trypanosome throughout the course of a "pure line" infection demonstrates very clearly that there are different periods of the infection and indicates the stage at which to make comparisons of size and variability between different infections. Let us take, for example, the coefficients of variation and the means for total length in rats 116 and 105 which are shown in figure 2. The infection in rat 116 was started from a single trypanosome and rat 105 was inoculated from rat 116. In other words, although the infection in the first rat started from a single specimen and in the second rat from many specimens, all of the trypanosomes in both rats are descendants of the single organism injected into rat 116. We can make no determinations during the incubation period since no one knows where the organisms are at this time. Let us consider first the constants for rat 105. On the first day of the blood infection the mean length was 24.785 ± .423. This rose rapidly until the 5th day when it reached 30.108 ± .280. This rise continued gradually until it reached 31.412 ± .065 by the 19th day. From the 19th until the 32nd day, at which time the infection disappeared from the blood, there was no significant change in the mean. The coefficient of variation behaved in much the same manner as the mean. On the first day of the infection it was 26.52 ± 1.35%. It dropped very rapidly for the first seven days and then much slower for the next twelve days. By the 19th day it reached the low value of 3.11 ± .14% and it showed no significant change from this value throughout the remainder of the infection.

It is well to compare the conditions found in rat 116 with those in rat 105. Rat 116 is given here because a longer time elapsed before the infection reached the adult stage than was the case in any of the other infections studied. This is due probably to the fact stated above, viz., that the infection in rat 116 started from a single trypanosome while the others although they were "pure lines" were sub-inoculated from 116, and in consequence were started with a large number of specimens. As these curves are probably expressions of the resistance of the host to the parasite, we would expect that this resistance would increase more rapidly when the infection is started with a large number of trypanosomes than when it is started with only one. We cannot compare the shape of the curves in the two rats very well because there are not enough points in the curve for rat 116. One thing is probably true, however, and that is that both the mean and the coefficient of variation reach a constant value by the 25th day. At first it looks as if the mean tends to rise even until the 32nd day. That this is not the case, however, is shown by the fact that the value on the 25th day does not vary appreciably from that of the 72nd day.

The same type of result was obtained for all of the six distances shown in figure 1, namely, for the distances posterior end *to* parabasal body, parabasal body *to* nucleus, nucleus *to* anterior end, anterior end *to* end of flagellum, and width, although most of the constants do not reach as low a level as is the case with those for total length (see table 1). Curves of this nature were obtained in four rats. These results prove, what we had been led to believe from cytological evidence, viz., that there is practically no division or growth in T. lewisi after the 25th day of the blood infection.

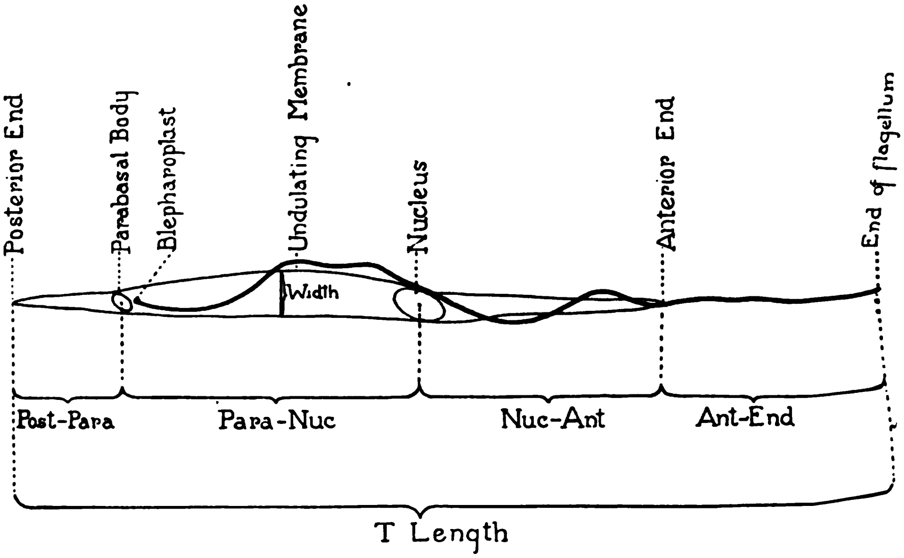

FIG. 1

Diagram of T. lewisi showing the parts of the organism and the distances measured in this work. The parts of the organism are placed vertically while the distances are placed horizontally. The latter consist of the distance from (1) posterior end to parabasal body, (2) parabasal body to nucleus, (3) nucleus to anterior end, (4) anterior end to end of flagellum, (5) total length, and (6) width.

After it was determined that there was no significant change in the mean or coefficient of variation after the 25th day we decided to make all measurements after this day. Most of the measurements which will be given in a later report were made on the 30th day of the blood infection. The fact that the trypanosomes in the blood of the rat reach what we can consider an adult stage makes size a very favorable character with which to work. It affectively eliminates growth factors from our computations.

The most interesting feature in the study of the variability exhibited by the "pure line" is that once the organism has reached the adult stage of infection the coefficients of variation are extremely low. Table 1 shows the constants for rat 105. The coefficients of variation for total length and parabasal-nucleus are 2.80% and 2.21%, respectively. It is to be noted also that these two characters show a lower coefficient of variation than any of the others. After considering this subject in great detail we have come to the conclusion that these two characters give the truest index of the variability in the "pure line." While there is not

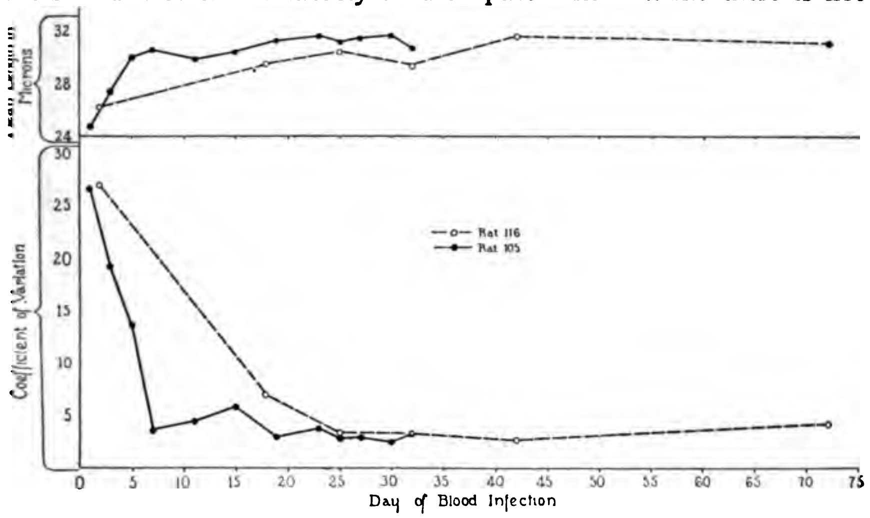

FIG. 2

raph showing the changes in the mean and coefficient of variation for total length in rats 116 and 105. The infection in 116 was started from a single organism and 105 was inoculated from 116. The trypanosomes disappeared from the blood of rat 105 on the 32nd day and from 116 on the 72nd day.

uch doubt that posterior-parabasal is more variable than either of ese two, we feel that much of its variability as well as that of the other stances with high coefficients is due to the difficulty of making the asurements.

Conclusion.—We can draw the following conclusions from this part the work: Trypanosoma lewisi reaches an "adult" stage in its development in the rat in about 25 days after it appears in the blood. Once this age is reached there is practically no division or growth. Due to the imination of growth factors, the organisms show a very low coefficient variation in "pure line" infections, provided they are measured after he "adult" stage is reached. These facts make T. lewisi a very favorable organism in which to study size and variation.

TABLE 1

CONSTANTS FOR THE SIX DISTANCES GIVEN IN FIGURE I FOR THE "PURE LINE" IN RAT 105 ON THE 25TH DAY OF BLOOD INFECTION. THE MEANS AND STANDARD DEVIATIONS ARE IN MICRONS

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Post-Para	4.268 ± .036	.544 ± .025	12.74 ± .61
Para-Nuc	10.854 ± .016	.240 ± .011	2.21 ± .10
Nuc-Ant	9.511 ± .047	.704 ± .033	7.41 ± .35
Ant-End	6.619 ± .068	1.010 ± .048	15.27 ± .74
T. Length	31.251 ± .059	.875 ± .041	2.80 ± .13
Width	1.590 ± .015	.230 ± .010	14.47 ± .70

[1] This and a later report form a preliminary account of a series of investigations which are being carried out in this laboratory on variation and inheritance in T. lewisi.

[2] Throughout this work the term "pure line" infection has been used to designate an infection, the trypanosomes of which have all arisen from a single organism. A given "pure line" may either have been started from a single specimen or it may have been subinoculated from such an infection.

[3] See especially Jennings, H. S., *Proc. American Phil. Soc.*, **47,** 1908 (393–546). *American Nat.*, **43,** 1909 (321–337). *Ibid.*, **45,** 1911 (79–89).

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VARIATION AND INHERITANCE IN SIZE IN TRYPANOSOMA LEWISI

II. THE EFFECTS OF GROWING "PURE LINES"[1] IN DIFFERENT VERTEBRATE AND INVERTEBRATE HOSTS AND A STUDY OF SIZE AND VARIATION IN INFECTIONS OCCURRING IN NATURE

By W. H. TALIAFERRO

DEPARTMENT OF MEDICAL ZOÖLOGY OF THE SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY

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In our first report[2] we demonstrated that Trypanosoma lewisi reaches an adult stage in the course of the infection in the rat in about 25 days, and once this stage is reached there is practically no division or growth. In consequence of the elimination of growth factors from the computations it was found that the coefficient of variation in the "pure line" was very low if it was computed during this adult period. The questions which now arise are: (1) Does growing the same "pure line" in different vertebrate hosts cause significant differences in the mean or the coefficient of variation? (2) Does passage of the "pure line" through the invertebrate host cause any significant differences in these constants? Our attention will be directed especially toward the coefficient of variation as this will give us an opportunity to ascertain whether or not there is a splitting up of the "pure line" into heritably diverse lines following such passage through the invertebrate host. With this background our final question is: (3) Does an infection occurring in nature consist of a large number of "pure lines" such as have been described in free-living protozoa, which differ among themselves but are *per se* constant in size?

It is to be emphasized at this point that in all of the following work the measurements and computations were made on or after the 30th day of the blood infection. As the adult stage is always reached by the 25th day this is well into the adult period and effectually eliminates growth factors. The six measurements of size used in this work have already been given in our first report (see figure 1). They consist of the following distances: (1) Posterior end *to* parabasal body, (2) parabasal body *to* nucleus, (3) nucleus *to* anterior end, (4) anterior end *to* end of flagellum, (5) total length which includes measurements 1–4, and (6) width.

Effect of Growing the Same "pure line" in Different Vertebrate Hosts.—The twoconstants in which we are interested in this connection are the size as indicated by the mean and the variability as indicated by the coefficient of variation. In all of the experiments the constants were worked out for all six measurements but in this brief report we will only deal with those for total length. Let us first consider the mean. The questions are: Does growing the same pure line in different *individuals* of the same species of rat produce significant differences in size? Does growing the same "pure line" in different *species* of rats produce any greater differences in size than in the first case of individuals belonging to the same species? It would have been interesting to grow the "pure line" in other vertebrates as well as in rats but T. lewisi is not normally infective to any other vertebrate. To test the first of these questions we used individuals of the albino rat and for the second question we used individuals of the black and the Norway rat. At the outset it may be definitely said that the differences which were obtained on growing the "pure line" in different species of rats were in no way more significant than the differences obtained by growing the "pure line" in different individuals of the same species. The greatest difference in mean size[3] was obtained when the same "pure line" was grown in the albino rats 46 and 90. In the former the mean length was 32.080±.052 and in the latter 30.769±.057, which gives us a difference of 1.311±.077. The mean lengths obtained in twelve other experiments lie between these limits. While a difference of this character, which is 16.8 times its probable error, is undoubtedly significant the question immediately arises as to whether such a difference is due to differences in the blood of different rats or to a personal variation in making the measurements. A definite answer cannot be given to this question at the present time although experiments are being carried out to ascertain, if possible, which is the determining factor. As, however, this is the greatest difference obtained in twelve experiments, we may say that growing the same "pure line" in different rats probably causes significant differences in mean size, but in any case these differences are small. Even if these differences are significant there is no evidence, at present, that they are due to inherited diversities, but are simply due to differences in environment.

In regard to variability, the chief thing of interest is the fact that passage of the "pure line" from rat to rat by blood inoculation has no effect on the coefficient of variation. Take, for example, the following experiment. The coefficient of variation for total length in the "pure line" as grown in rat 116 was 2.77±.13%. After four passages through white rats this line was grown in rat 204 where the coefficient of variation was found to be 2.60±.12%. The difference between these two coefficients is .17±.18% which certainly is not significant. Several other experiments gave similar results and an experiment is being carried out now in which the "pure line" is undergoing numerous transfers from one rat to another. The fact that passage of the pure line through rats has no effect on the coefficient of variation is in marked contrast to conditions found after passage through the invertebrate host.

Effect of Passing the "pure line" through the Invertebrate Host.—While T. lewisi can be experimentally transmitted by several species of ectoparasites the natural insect vectors are the rat fleas, Xenopsylla cheopis and Ceratophyllus fasciatus. In working out the life history of certain species of trypanosomes some of the earlier investigators described what they considered to be sexual phases in the life history of the parasite. Practically all of these observations are open to entirely different interpretations, so that at the present time, although a number of protozoölogists feel that there is a sexual phase in the life cycle, no such phase has been demonstrated. This is notably true in the classic researches of Minchin and Thomson[4] on the life history of T. lewisi in the rat flea. Although they looked carefully for conjugation they found no evidence of it.

While there is no evidence of sexual phenomena in the trypanosomes during passage through the invertebrate host, there is evidence that such passage exerts a profound effect on such things as acquired physiological characteristics. Gonder[5] for example found that arsenic fastness was transmitted from rat to rat but was lost by passage through the louse. Miss Robertson[6] found that strains of T. gambiense showed marked changes in their characteristics after passage through the tsetse-fly. This led this author to say, "It seems clear that the cycle in the fly as a whole, whether conjugation occurs or not, has much of the biological significance of the process."

As we have seen passage of the same "pure line" through a number of rats does not increase the variability of the "pure line." Passage through the flea, on the other hand, *invariably* increases the variability. These experiments were carried out in the following manner. Fleas which were carefully raised in the laboratory and which were known to be free from infection were allowed to bite animals infected with a given "pure line." After about a week (during which time the trypanosomes were undergoing their development in the flea) other rats were infected either by teasing up a single flea and injecting it intraperitoneally or by allowing the rat to lick up the moist feces, which is the natural mode of infection. The trypanosomes in the rat which was infected from the flea were measured on the 30th day of the blood infection. Six such experiments have been successfully carried out. The following is a fair example of all of them. The pure line in rat 105 showed a coefficient of variation of 2.80 ± .13% for total length. After passage through a single specimen of X. cheopis it was measured in rat 163. Here the coefficient of variation increased to 5.24 ± .25%, a difference of 2.44 ± .28%. As we have seen above, no such increase in variability has been observed during passage of the "pure line" from rat to rat. From these experiments we must conclude that the "pure line" breaks up during passage through the invertebrate host. This is analogous to the results of Jennings[7] in Paramecium after conjugation and in a recent publication Miss

Erdmann[3] believes that she has demonstrated the same type of phenomena in Paramecium after endomixis. While the increase in variability is probably due to some nuclear phenomena during the life cycle of the trypanosome in the flea, we have no method of judging, from the present data on the subject, whether it is a sexual or a reorganization process. Minchin and Thomson[4] have suggested that the peculiar effects produced by passage through the invertebrate host are connected with the transformation of the trypanosome into a crithidial stage and the reverse process, both of which always take place in the life cycle in the invertebrate host.

It is conceivable that passage of a "pure line" through the invertebrate host might cause significant changes in the mean size as well as in the variability. All of our experiments have shown this not to be true. It is true that after the "pure line" is passed through a flea there is often a difference in the mean which is significant from a statistical standpoint, but as these differences are never greater than the maximum difference given above for growing the "pure line" in different rats, we cannot ascribe the difference to any peculiar effect of the flea. It is probably due simply to the fact that the trypanosomes are measured in different rats.

Size and Variability in "wild"[1] *Infections Occurring in Nature.*—A number of naturally infected rats were collected from around Baltimore and Washington. Up to the present time measurements have been made of specimens from ten of these infections. While these "wild" infections do show differences in their means and a higher coefficient of variation on the average than the pure lines, one is struck with the constancy of the means and the low variability. The longest mean length (rat 221) obtained is 32.503±.060 and the shortest (rat 411) 29.093±.062 with a difference of 3.410±.087. It can be seen that the difference between the longest "wild" infection and the shortest is comparatively great and that the difference is much greater than is obtained by growing a "pure line" in different rats. The least variable "wild" infection (rat 413) showed a coefficient of variation of only 2.125±.101. This is slightly lower than any of the coefficients of the "pure lines" in the laboratory. The most variable "wild" infection (rat 65) showed a coefficient variation of 4.583 ± .223 The difference between the least and the most variable "wild" infection is 2.45 ± 24. The remainder of the "wild" infection measure fall between the figures given here, both as regards their means and coefficients of variation.

From these results we must conclude that while the "wild" infections occurring in nature show comparatively small differences in size, these differences are greater than can be explained by the fact that the infections are occurring in different rats. We must also conclude that most "wild" infections consist either of very few "pure lines" or a larger number which differ *inter se* by very minute differences. In some cases, as in that of rat 413, we are almost forced to believe that a given "wild" infection is actually a "pure line." A possible explanation of these facts suggests itself when we consider the mechanism of infection with T. lewisi. As stated above the rat receives the infection from the flea. As the infected flea is feeding it deposits its feces, which contain the infective forms of the trypanosomes, on the fur of the rat. The rat then becomes infected by licking up the *moist* feces containing the infective forms. In nature it is probable that a given rat receives its infection from a single flea. This flea deposits a number of infective forms. The chances are that most of these will be killed by drying and that only a few will actually start the infection in the rat. This being so, the variability of the infections simply reflect the number of infective forms of the trypanosomes that actually start growth in the rat. In fact it is conceivable that a number of the "wild" infections are actually "pure lines."

At the present time the author is of the opinion that if we consider all the infections in nature collectively we can say that there are a large number of "pure lines" which differ among themselves but which (as is shown by work in the laboratory) are *per se* constant in size. Due to the peculiar way in which rats receive their infections, however, we find that any given infection consists of only a very few of these "pure lines."

Conclusions.—At the present phase of the work the following conclusions may be drawn: While growing the same "pure line" in different rats may cause significant differences in the mean size, these differences are small. The differences in the mean are never greater when the "pure line" is grown in different species of rats than when it is grown in different individuals of the same species. Passage of the "pure line" from rat to rat is not followed by any significant changes in the coefficient of variation. In marked contrast to this it is found that passage of the "pure line" through the flea is invariably followed by a significant increase in the coefficient of variation which is interpreted as showing that the "pure line" breaks up into heritably diverse lines following such passage. Although passage through the flea has such a marked effect on the variability it has no significant effect on the mean size. "Wild" infections occurring in nature exhibit differences in their mean sizes which are greater than can be explained by the fact that they occur in different rats. The coefficients of variation of "wild" infections are, on the average, higher than for the "pure line." The lowest of these coefficients, however, is no greater than the coefficient of variation for the "pure line" and even the highest can be explained on the assumption that the "wild" infection consists of only a very few "pure lines." It seems probable that there occur, in nature as a whole, a large number of "pure lines" which differ among themselves but are *per se* constant in size, but, due to the peculiar way in which rats receive their infections, a given infection consists of only a very few of these lines. In fact, a large number of "wild" infections are probably actual "pure lines."

[1] Throughout this work the term "pure line infection" is used to designate an infection, the trypanosomes of which have all arisen from a single organism. A given "pure line" may either have been started from a single trypanosome, or it may have been subinoculated from such an infection. The term "wild infection" designates an infection as found in nature.

[2] These PROCEEDINGS, 7, 1921 (138-143).

[3] The mean sizes in this report are all in microns.

[4] Minchin, E. A., and Thomson, J. D., *Quart. J. Microsc. Sci.*, 60, *N. S.*, 1915 (463-692).

[5] Gondor, R., *Centralbl. Bakt., etc.*, I abt., Originale, 612, 1911 (102-113).

[6] Robertson, M., *Proc. Roy. Soc.*, (B) 85, 1912 (241-248).

[7] Jennings, H. S., *J. Exper. Zool.*, 11, 1911 (1-134); *Ibid.*, 14, 1913 (270-391).

[8] Erdmann, R., *Archiv. Entwicklungsmech. Organ.*, 46, 1920 (85-148).

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this description is that he described, in the nucleus of the cysts, besides a large eccentric karyosome, a crescent-shaped mass of chromatin granules. We will take up a discussion of this description later.

In the same year Kofoid, Kornhauser and Swezy (1919) came to the conclusion that Wenyon and O'Connor's " iodine cysts " include two different organisms. They came to the conclusion that the homogeneous bodies seen by these investigators and considered by them to be " iodine cysts " devoid of their iodophilic inclusions are " the non-nucleated, homogeneous spore-like cysts of some vegetable organism, possibly the chlamydospore of some phycomycete of unknown affinities," and that the large glycogen-laden " iodine cysts " are uninucleate cysts of *Endolimax nana.* It is not necessary to consider these authors' conclusions in regard to the homogeneous bodies included by Wenyon and O'Connor in the " iodine cysts," because we feel that these bodies are probably not typical " iodine cysts." It is with the second conclusion that we are most interested, namely, that the " iodine cysts " are the uninucleate cysts of *Endolimax nana.* As we understand it, these authors base this conclusion on the following points: In the first place, it is known that the common intestinal amoebae (*E. histolytica, E. coli,* and *E. nana*) often have large glycogen masses during the early one- and two-nucleate stages of their cysts, and if the " iodine cysts " are, in reality, uninucleate cysts of *E. nana,* it is not surprising to find a large glycogen mass in them. In the second place, they maintain that the nucleus of the " iodine cysts " is similar to that of an uninucleate *E. nana* cyst in respect to: (*a*) dimension of nucleus, (*b*) position of nucleus in cyst, (*c*) structure, both as regards nuclear membrane, and arrangement and distribution of the chromatin. And in the third place, they maintain that there is an intergradation in size of the glycogen mass found in the " iodine cysts " and in uninucleate cysts of *E. nana.* Besides the question of the structure of the nucleus, which will be discussed in detail in a later section, there are two difficulties in the way of accepting the conclusion of these authors. The first of these is that the " iodine cysts " are, on the average, larger than those of *E. nana;* and the second is that these authors were unable to find any of the larger cysts with four nuclei which should occur, if these were cysts of *E. nana.* As a matter of fact, the great majority of the " iodine cysts " are uninucleate, and it is only rarely that a specimen with even two nuclei is seen. That these difficulties were clearly

fixation and available stains, to demonstrate in the nucleus of *C. lafleuri,* the eosinophilic granules which Dobell describes for the amoeba of the " iodine cysts." They even suggest that, with this exception, there is enough in common with their description of *C. lafleuri* and Dobell's description of *Iodamoeba* to make it desirable to reinvestigate Dobell's *Iodamoeba* to see whether or not it should not be considered *C. lafleuri.* It is, however, a little difficult to see their grounds for this suggestion, as the range of size of the two species is not at all similar—*Councilmania* being a very large amoeba and *Iodamoeba* being a very small form.

Brug (1921) has also returned to the study of the amoeba of the " iodine cysts." His description of the amoeba and its cysts is essentially the same as that given in his earlier paper. He also still feels that the amoeba should be called *Endolimax williamsi* Prowazek, 1911. In regard to the nomenclature he has one very interesting new fact to offer, in that he has been able to examine Prowazek's original slides of " Entamoeba williamsi." He finds that these slides contain both *E. coli* and the amoeba of the " iodine cysts." He feels, therefore, that although *E. bütschlii* Prowazek, 1912, is entirely the amoeba of the " iodine cysts," *E. williamsi* Prowazek, 1911, is in part the same species, and that the latter name should have precedence over the former.

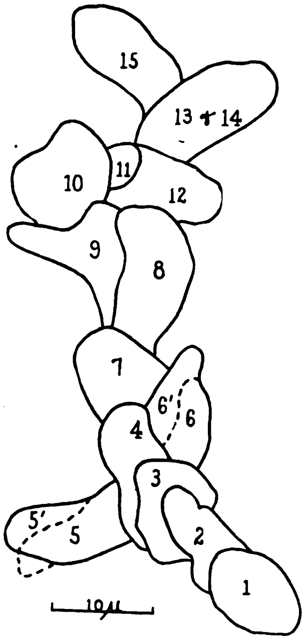
Nöller (1921) has been able not only to examine Prowazek's original slides of *E. williamsi,* but also of *E. bütschlii.* This examination supports the contentions of Brug. Nöller feels, however, that since Prowazek's description of *bütschlii* is based solely on the amoeba of the " iodine cysts," whereas the description of *williamsi* is based only in part on this amoeba, the sensible thing is to accept the former name. He therefore uses the name, *Iodamoeba bütschlii* Prowazek, 1912, emend. Dobell, 1919.

Besides the work cited above, Rodenhuis (1919) has proposed the name *Endolimax pileonucleatus* for the amoeba in question. The author's grounds for proposing this name are not clear. One thing of interest in this paper is that the author has found and described what he believes to be several stages in the division of the nucleus. In the paper in which Chauchemez (1921) describes the finding of " iodine cysts " in the faeces of pigs, he notes that Brumpt (personal communication) accepts Dobell's genus *Iodamoeba,* but because he does not believe that the amoeba can be identified with any previously described species, he (Brumpt) proposes the name *Iodamoeba wenyoni.*

THE HUMAN INTESTINAL AMOEBA, IODAMOEBA WILLIAMSI, AND ITS CYSTS (IODINE CYSTS).

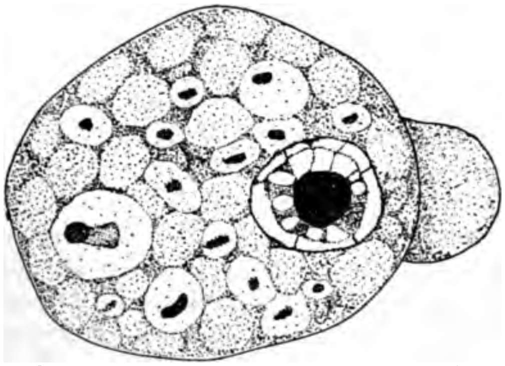
other characteristics of the species which should be mentioned. According to Dobell (1919) the diameter of this amoeba, when rounded, is usually from 9 to 13 μ. He also observed larger specimens up to 17–20 μ and smaller ones of 5 μ in diameter. Kuenen and Swellengrebel (1917) give the diameter as 10–12 μ and Brug (1921) states that in his experience it is 7 to 20 μ. The maximum size which Prowazek (1912) gives for his *Entamoeba bütschlii,* which is probably the same species, is 24 μ. As Dobell (1919) points out, however, Prowazek may have mistaken a small specimen of *E. coli* for the present form. In our work we find that fixed and stained specimens of the motile amoeba, when rounded, vary in diameter from 9 to 14 μ, the most usual diameter being 11 μ. The largest specimen which we have seen was 20 by 15 μ. In this connection it is interesting to note that, in our experience, the active amoebae are on the average about 2 μ greater in diameter than the cysts. This indicates that the amoeba undergoes a reduction in size just before encystment. Such a process is known to occur in the other intestinal amoebae, but Dobell (1919) maintains that it is not the case in the amoeba of the "iodine cysts."

When the motile amoebae are examined in the living condition on a warm stage, their movement is very similar to that of a small *E. coli.* The rapidity with which different specimens move varies greatly, some being very sluggish and others moving with great rapidity. Text-figure 1 represents a series of camera lucida outlines of a rapidly moving specimen drawn at intervals of 15 seconds. In such specimens there is usually no sharp distinction between ectoplasm and endoplasm. Occasionally, however, a specimen will stop its progressive movement and send out a pseudopodium which is sharply marked off from the granular body of the animal. These clear hyaline pseudopodia are formed very rarely, and we have never seen them used as organs of locomotion. We believe that they represent an abnormal condition, and that the formation of the *coli*-type of pseudopodium is the normal method of progression. In contrast to such active specimens as the one shown in Text-fig. 1, we have often observed specimens for as much as 15 minutes which constantly threw out pseudopodia and yet scarcely moved from their original position. The visibility of the nucleus in the living amoebae depends largely on the amount of the food inclusions. Specimens are often seen which contain numerous bacteria and almost every type of intestinal debris, as in the specimen shown in Fig. 1, Plate VIII. Such specimens, when alive, remind one of *E. coli,* but the nucleus is rarely visible in them. In other specimens which contain little or no food, the nucleus with its large central karyosome can often be clearly seen.



TEXT-FIG. 1. Series of camera lucida outlines of a very active specimen of *Iodamoeba williamsi* which shows the characteristic changes in shape in such specimens. The specimen was observed on a warm stage, and the outlines were made at 15 second intervals. Numbers 1–15 indicate the shape and position of the amoeba at each consecutive interval.

The size of the nucleus varies with that of the amoeba, being roughly about one quarter the diameter of the entire specimen. Its structure can be considered under four heads: (1) the nuclear membrane, (2) the karyosome, (3) the peripheral layer of granules ("peripheral chromatin"), (4) the linin threads which extend from the karyosome to the nuclear membrane. The nuclear membrane is clearly visible in any of the stains used and often contains granules or even small lumps of deeply staining substance. Whether or not these masses are chromatin cannot be said at the present time. The large karyosome is, in our experience, always located centrally in the nuclei of the motile forms. Its diameter is roughly about one half that of the nucleus (see Plate VIII, Figs. 1–4). When stained with haematoxylin stains, it often is not homogeneous in structure, but exhibits a lighter central area. When stained with Mann's stain, there are often present one or several darker staining granules in the center. Figure 3, Plate VIII, shows a specimen which contains three of these structures. The nature of these structures is not clear—certainly there is no valid reason for assuming them to be centrioles, as is done by Prowazek (1912) in his description of "Entamoeba bütschlii," which is probably the same form. Immediately surrounding the karyosome, there is a layer of granules which we have termed the peripheral granules and which is often termed "peripheral chromatin." In a large number of the specimens stained with either iron haematoxylin and eosin, or with Mann's stain, this layer of peripheral granules appears as a simple layer divided into segments by numerous radiating septa (Plate VIII, Figs. 1 and 2). In well-stained specimens, however, it can be seen that this is not the true structure of this layer, but that each segment is in reality an ovoid granule embedded in a deeper staining matrix. (Plate VIII, Figs. 3 and 4.) In the most favorable specimens, with careful focusing of the microscope it is even possible to see that each of these granules has a slightly higher refractive index than the surrounding protoplasm. This layer of peripheral granules is sharply differentiated from the karyosome, in material stained in Mann's stain—the layer of granules staining blue and the karyosome red. The appearance in iron haematoxylin and eosin depends largely on the extraction of the haematoxylin. As pointed out by Dobell (1919) the granules take both nuclear and plasma stains, but give up the former much more readily than the karyosome. Not only is this true in our experience, but we have also found that the matrix in which the granules are embedded is intermediate in this respect—decolorizing much sooner than the karyosome, but retaining the stain (haematoxylin) longer than the actual granules It is, therefore, possible to stain the entire granule layer black like the karyosome, or to decolorize the nucleus until the actual granules are colorless, but the matrix around them is still dark (Plate VIII, Fig. 3), or to decolorize the nucleus to such an extent that the entire granule layer—including both the actual granules and their surrounding matrix—appears practically colorless, with the karyosome still retaining an almost black stain. In the latter case the entire granule layer can be restained with eosin. While the above description of the way in which the granules behave with haematoxylin stains is true in general, one finds that each granule may show some idiosyncrasy in the way in which it retains the stain. Such a condition of affairs is shown in the nucleus of the specimen in Text-fig. 2. Here some of the granules have completely given up the stain, while others cannot be distinguished from the matrix in which they are embedded. We shall see later that this variability with which the different granules take or give up haematoxylin probably explains some of the peculiar pictures which are often seen in the nuclei of cysts.



TEXT-FIG. 2. Camera lucida drawing of a motile specimen of *I. williamsi*, stained with iron-haematoxylin. Note the peculiar way in which some of the peripheral granules around the karyosome are stained while others are not. Compare with Figs. 1–4, plate VIII. × 4,300.

In the majority of our specimens fixed with Schaudinn's fluid and stained with either iron haematoxylin and eosin or with Mann's stain, it is possible to distinguish several fine linin strands running from the karyosome to the nuclear membrane. These linin fibers must not be confused with the appearance of septa in the granule layer noted above. These linin fibers extend through the granule layer and actually touch the nuclear membrane (Plate VIII, Figs. 1–4). Dobell (1919) does not mention these strands, but they are noted by Brug (1921). It is very difficult, however, to interpret Brug's figures. We are unable from a study of his figures of the motile form to decide whether he has decolorized the layer of peripheral granules to such an extent that a few "septa" are left, or whether he has overstained them so that the layer of granules is not differentiated from the karyosome, in which case the strands shown would be the linin strands in question. Judging from the comparative size of the karyosome to the entire nucleus, we feel that the latter is probably the true explanation of his figures.

differences between the foregoing account and the figures given by Brug (1921) are to be explained by an overstaining of his specimens with haematoxylin. In such overstained specimens, the layer of peripheral granules cannot be differentiated from the karyosome, and this gives the nucleus the appearance of containing a large central karyosome, connected to the nuclear membrane by a few linin strands.

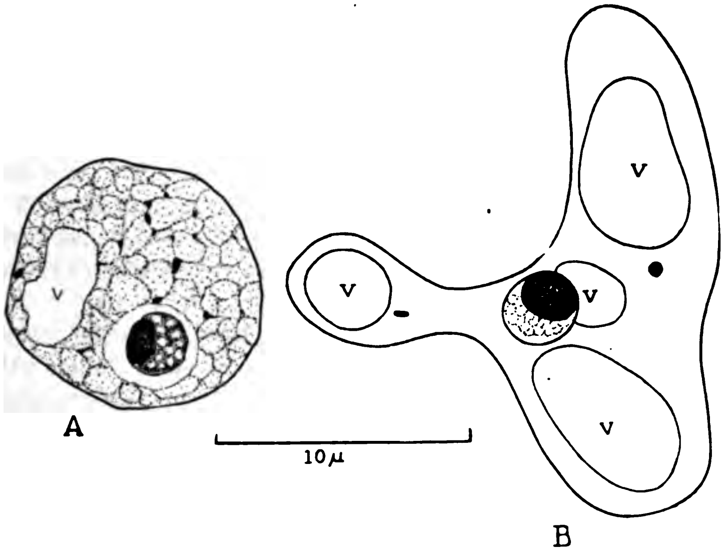
THE CYST.

It is difficult to give a single measurement of size for the cysts, due to the fact that so many of them are irregular in shape. To overcome this difficulty in the case of the irregular cysts, Dobell (1919) has taken the average of the greatest length and the greatest width, as the "size." In using this measure of "size" which we have found very valuable, we have found that most cysts lie between 6.4 μ and 16.6 μ, and that the average is about 9.1 μ. In our experience, the irregularity in shape of the cysts varies considerably from day to day in the same infection. Some days almost all of the cysts are nearly spherical, and on other days many are very irregular in shape. In this connection mention should be made of a most remarkable structure which we found on one of our prepared slides, which is undoubtedly an "iodine cyst." A camera lucida outline of this cyst is given in Text-fig. 3 *B*. By comparing this cyst with the one beside it, which is of ordinary size, it can be seen that it is abnormally large and irregular in outline. This monster contained four glycogen vacuoles. In spite of its abnormalities, however, the structure of the nucleus leaves no doubt that it is a true "iodine cyst." In our work we have not found any other cysts which were comparable to this one, either in size or in shape.

It is not necessary to describe the appearance of the cysts, when examined in the living state, or in iodine solution, as this has been done by a number of investigators. We can, therefore, pass directly to a consideration of the nuclei of the cysts. The mature cysts practically all contain a single nucleus. Occasionally supernucleate cysts, with two nuclei, are found, but these are rare. In order to ascertain approximately the percentage of the cysts that are binucleate, we examined 2,000 cysts consecutively, as they appeared on some of our prepared slides. Among those 2,000 cysts only four contained two nuclei—giving a percentage of 0.2. As far as we can find, no investigator has described cysts with more than two nuclei.

With the exception of the presence of the fine linin fibers extending from the karyosome though the layer of peripheral granules to the nuclear membrane, the foregoing account agrees, almost in detail, with the description given by Dobell (1919). We believe further that the

The nucleus of the cyst possesses the same structures which were described for that of the motile form, viz., (1) a nuclear membrane, (2) a karyosome, (3) peripheral granules (" peripheral chromatin "), and (4) linin strands running from the karyosome to the nuclear membrane. Structures (1) and (4) are the same as in the motile amoebae, but the position and relationship of (2) and (3) are greatly modified, when the amoeba encysts. These changes can readily be seen by comparing the nuclei of the motile forms, shown in plate VIII (Figs. 1–4), with those of the cysts, shown in the same plate (Figs. 5 and 6). The karyosome, instead of being central, as it is in the motile amoeba, becomes eccentrically placed, and the layer of granules, instead of surrounding the karyosome, becomes massed to one side of it. The number of these granules varies greatly. Sometimes they are several layers deep and completely fill the nuclear cavity on one side of the karyosome, and at other times, they are comparatively few in number and are closely applied to one side of the karyosome (Plate VIII, Figs. 5 and 6). Of course the actual appearance of the nucleus



Text-fig. 3. A, typical cyst of *I. williamsi* with a single glycogen vacuole, *v*. B, unusually large and irregular cyst containing four glycogen vacuoles, *v*.

varies according to the pole from which it is viewed. If it is seen from the pole toward which the granules are displaced, the contour of the karyosome is obscured, giving a picture like the one shown in Plate VIII, Fig. 7. If, on the other hand, it is seen from the opposite pole, i.e., the pole toward which the karyosome is displaced, the latter is seen surrounded by the granules (Plate VIII, Fig. 8). In comparing these two figures (Plate VIII, Figs. 7 and 8), it must be kept in mind that they differ markedly in another respect, viz., the number of granules present in each.

As in the case of the motile amoebae, our description of the structure of the nucleus of the cysts agrees, almost in detail, with that of Dobell (1919). Dobell, however, does not note or figure the linin strands, running from the karyosome to the nuclear membrane, which are shown in figure 6, and, to a less extent, in Fig. 5, Plate VIII. Most of our material, which was fixed in Schaudinn's fluid and stained in either iron haematoxylin and eosin or in Mann's stain, showed these strands. They were, however, absent in some of the cysts, as is shown in Text-fig. 3 *A*. Incidentally this figure is very similar to some of those given by the above investigator.

While our results are in accord with those of Dobell, they are not in agreement with those of Brug (1919 and 1921), Nöller (1921), or Kofoid, Kornhauser, and Swezy (1919). Let us first consider the findings of Brug. In his earlier paper (1919) he describes the nucleus as containing a large karyosome and a crescent-shaped mass of chromatin granules which is separated from the karyosome by a considerable space. This description is repeated in his later paper (1921). It is very difficult to ascertain the exact nature of this crescent-shaped mass of chromatin granules from his description. One would be led to think that the author means that the crescent-shaped mass is composed of chromatin of the same nature as found in the karyosome. Certainly, there is no evidence that he means anything like the peripheral granules which we have just described, and which show an entirely different staining reaction from the karyosome when stained with Mann's stain. This interpretation is further supported by the fact that the author places the amoeba in the genus *Endolimax*—presumably on his interpretation of the structure of the nucleus. Another thing of interest is that the author apparently believes that the crescent-shaped mass of chromatin granules arises only after the amoeba encysts, whereas the peripheral granules, which we have just described, were represented in both the free forms and in the cysts.

We were, at first, unable to explain Brug's figures. Later on in our work, however, we obtained preparations which appear so similar to his that we feel that we have an explanation of his findings. In some of our material which was fixed with Schaudinn's fluid and stained with iron haematoxylin, we obtained many specimens similar to those shown in Plate IX, Figs. 9 and 10. These specimens show the presence of a crescent-shaped mass widely separated from the karyosome, and are almost identical with some of Brug's figures. One thing is at once apparent, however, viz., the crescent-shaped mass is composed of the same large granules ("peripheral chromatin") which Dobell and the present authors have described, as occurring between the karyosome and the nuclear membrane. The mass itself often stains as black as the karyosome, due to the way in which the matrix around the granules is stained. Why, then, should this mass of granules be so widely separated from the karyosome in this material, and not in the material described above? We first thought that it was because of shrinkage due to improper fixation. This is hardly the case, however, because in another set of the same slides, fixed in the same manner at the same time, but stained in Mann's stain, we could trace the granules clear up and in contact with the karyosome. It seems more probable, therefore, that the appearance of the crescent-shaped mass is to be explained on the assumption that the layer of granules next to the karyosome often gives up the haematoxylin stain more readily than the remaining granules. This assumption is supported by the fact that in haematoxylin specimens one can often see the granules extending to the karyosome, but those nearest to the karyosome are much more lightly stained than those further away. Another fact may enter into the explanation, and that is the question of the matrix in which the granules are embedded. This matrix undoubtedly takes the haematoxylin more readily than the actual granules, and the appearance of the separate mass may be due in some part to the presence of less matrix near the karyosome than further away. At any rate it is interesting to be able to duplicate Brug's results and see that his crescent-shaped mass is composed of the same peripheral granules which we described above. Furthermore, we feel that it is probable that in such specimens, as are shown in Plate IX, Figs. 9 and 10, there is a layer of unstained granules between the karyosome and the dark crescent-shaped mass.

While Nöller (1921) does not give a detailed description of the nucleus, it is clear that he agrees with the description given by Brug.

He states in a footnote (page 41) that the characteristic nuclear structure can best be seen in Delafield's haematoxylin or in haemalum preparations, and that the half-moon (crescent-shaped mass) can be seen only poorly in iron-haematoxylin preparations. He also states that not a single one of Dobell's figures depicts the true nuclear structure of the cysts, due to the fact that differentiation has been carried too far. In regard to Nöller's first statement we can say that we have been able to get this structure in iron haematoxylin as well as in Delafield's haematoxylin. We also believe that the second statement is based on a misinterpretation of the facts. It implies that the crescent-shaped mass is something different from the granules described by Dobell. As we have shown above, however, it is in reality composed of the same granules which Dobell shows in his figures, and which we have illustrated in Plate VIII, Figs. 5 and 6. Furthermore, we feel that the mass of these granules extends to the karyosome, and that there should be no space between them and the karyosome.

Besides the appearance of the crescent-shaped mass, many other peculiar nuclear pictures are often seen in iron-haematoxylin preparations. Some of these suggest very strongly the condition of affairs often met with in *E. nana,* and may offer an explanation of the results obtained by Kofoid, Kornhauser and Swezy (1919)—especially as these investigators used this stain almost exclusively. A few of these nuclei are shown in Plate IX, Figs. 11 to 16. In the case of the nucleus shown in Fig. 11, the mass of granules has been so poorly stained that the contour of none of them can be seen. Incidentally it may be remarked that this nucleus is viewed obliquely from the pole toward which the karyosome is displaced. In Figs. 12 and 14 a few of the granules themselves have apparently taken the haematoxylin stain. In Fig. 15 practically all trace of the mass of granules has been removed by destaining. The nucleus in Fig. 16 is very similar to that of *E. nana.* Here it seems probable that the larger black mass is the karyosome and the smaller one with the strand which attaches it to the larger mass represents the mass of granules. We do not know why some granules in the mass should decolorize so readily and others retain the stain, but we do know that the different granules often show many idiosyncrasies, in the way in which they behave, in the iron-haematoxylin process. We feel that, if any of these cysts had been stained in Mann's stain, we would have been able to demonstrate the karyosome and the peripheral granules, the former staining red and the latter blue, because some of the identical material, fixed at the same time, was stained in Mann's stain and did give these results. The above statements must not be construed to mean that accurate pictures of the nucleus are *never* obtained with iron haematoxylin. This stain, especially if used with eosin, often gives beautiful results, but it also often behaves as described above.

Our suggestion that the results of Kofoid, Kornhauser and Swezy can be explained in the above manner is not based on work with the " iodine cysts " alone. We have also examined a large number of both motile and encysted forms of *E. nana*. This was done to see if, with our technique, we could demonstrate the same type of nucleus in *E. nana* as we did in the " iodine cysts." In none of these specimens, however, have we been able to demonstrate anything comparable to the peripheral granules described above. The karyosome was always represented by one or several masses which were generally connected by a fine chromatic strand. A typical cyst of *E. nana* is shown in Plate IX, Fig. 17. All of our work, therefore, points to the conclusion that not only is the amoeba of the "iodine cysts" not identical with *E. nana*, but its nucleus is so different from that of the latter species, which is the type species of the genus *Endolimax*, that it cannot even be placed in the same genus.

*Nomenclature**

The nomenclature of the parasitic amoebae is somewhat confused at the present time. The correct parent genus is undoubtedly *Endamoeba* Leidy 1879, type by original designation *E. blattae*. Since the formation of this genus the parasitic amoebae have been divided into a number of genera, the distinguishing characteristics of which are chiefly differences in nuclear structure (see Dobell and O'Connor, 1921). If we accept this classification the amoeba of the " iodine cysts " cannot be placed in the genus *Endamoeba* Leidy 1879, type *E. blattae* (*Entamoeba* Casagrandi and Barbagallo, 1895, type *E. coli*), or *Endolimax* Kuenen and Swellengrebel 1917, type *E. nana*. We believe that Brug's contention that the amoeba is a species of *Endolimax* rests on a misinterpretation of the nature of the crescent-shaped mass. If this mass were composed of the same type of chromatin as the karyosome, the nucleus would resemble an *Endolimax* nucleus. This is not the case, however, because the crescent-shaped mass is, in reality, an aggregation of the same type of large granules which surround the karyosome in the motile stage. While these granules may be a kind of chromatin, they do not give the same staining reaction as the karyosome, and are probably of an entirely different nature. The contention of Kofoid, Kornhauser and Swezy that the " iodine cysts " are the uninucleate cysts of *E. nana* has already been considered. As the amoeba cannot be placed in the genus *Endolimax,* the first available name is *Iodamoeba* of Dobell (1919). Kuenen and Swellengrebel's (1917) name, " Pseudolimax," was proposed, distinctly as not subject to the rules of nomenclature, and hence not as a true generic name.

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* We arrived at the conclusions expressed in this section, particularly those in regard to the specific name, after a conference with Dr. C. W. Stiles. It is a great pleasure to express our indebtedness to Dr. Stiles for his helpful advice.

Nöller (1921) and Brug (1921) have both examined Prowazek's original slides and agree that *Entamoeba williamsi* Prowazek 1911 is a mixture of *E. coli* and the amoeba of the " iodine cysts." Practically all observers agree that the species which Prowazek described the next year under the name *Entamoeba bütschlii* is entirely the amoeba of the " iodine cysts." The question arises, then, as to which name should be used for the amoeba of the " iodine cysts." If *williamsi* Prowazek, 1911, is to be considered a synonym of *E. coli, bütschlii* Prowazek, 1912, is the first available name. If, however, it can be shown that *williamsi* Prowazek, 1911, is available for the present form, it must take precedence over *bütschlii* Prowazek, 1912.

Castellani and Chalmers, 1913, and Hartmann, 1913, were the first authors to consider *E. williamsi* Prowazek. The former investigators considered it a probable synonym of *E. coli* but the latter clearly indicated that he considered it different from *E. coli* because he designated it *Entamoeba coli* var. *williamsi.* We have, therefore, two revisors of the same year for Prowazek's species and there is no way of fixing precedence by any dates given in the publications. We are, however, justified in accepting Hartmann's revision because he *definitely* stated that he considered *williamsi* different from *coli* whereas Castellani and Chalmers (1913, p. 269) gave *williamsi* as a *probable* synonym of *coli.* Another fact which necessitates our acceptance of Hartmann's revision is that the first subsequent investigator to recognize the nature of the organism under question (Brug 1919) and to designate the type material (Brug 1921) followed the spirit of Hartmann's revision. It is very important to note that Hartmann based his distinction between *coli* and *williamsi* on the motile amoeba and, therefore, if it can be shown that the motile stage of *williamsi* is different from *E. coli* we will have to retain the name *williamsi* for this new species, and it will not be permissible to consider it a synonym of *E. coli.* Not only have Nöller (1921) and Brug (1921) shown that Prowazek's original slides contained both *E. coli* and the amoeba of the "iodine cysts," but an examination of Prowazek's description of *E. williamsi* shows that the motile stages of this form (Figs. 1 and 2) are those of the "iodine cysts." A further fact of paramount importance is that Brug (1921) clearly designated Prowazek's original slides as the type material of the species. The revision of Prowazek's *williamsi* by Hartmann (1913), the designation of Prowazek's original material as the type material of *williamsi* by Brug (1921), and the identification of this material with the amoeba of the "iodine cysts" by Brug (1921) and Nöller (1921), make it quite clear to us that under the International Rules of Nomenclature (Articles 29, 30, and 31) *williamsi* is the valid name of the species in question.

To sum up, then, we feel that the name of the amoeba of the "iodine cysts" should be *Iodamoeba williamsi* Prowazek 1911.

CONCLUSIONS.

1. In the nucleus of the motile amoeba of the "iodine cysts" there is a well-defined nuclear membrane, a large central karyosome, a single layer of peripheral granules ("peripheral chromatin") which surrounds the karyosome, and a few fine linin strands which run from the karyosome through the layer of peripheral granules to the nuclear membrane. The peripheral granules show a different staining reaction from the karyosome.

2. The nucleus of the cysts contains the same structures as that of the motile amoebae. The karyosome, however, becomes eccentrically placed, and the peripheral granules, which vary greatly in number among different nuclei, become localized on one side of the karyosome, between it and the nuclear membrane.

3. With the exception of the linin strands, our description agrees with that of Dobell. The crescent-shaped mass which is described by Brug and Nöller in the nuclei of the cysts, is composed of the granules noted above. In cysts which are stained with iron haematoxylin and decolorized to too great an extent we have found many pictures which remind one very much of *E. nana,* and may explain the findings of Kofoid, Kornhauser and Swezy.

4. The structure of the nucleus of the amoeba of the "iodine cysts" makes it impossible to identify it with *Endolimax nana* or even to include it in the genus *Endolimax*.

5. The name of the amoeba of the "iodine cysts" should be *Iodamoeba williamsi* Prowazek 1911.

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ERRATUM

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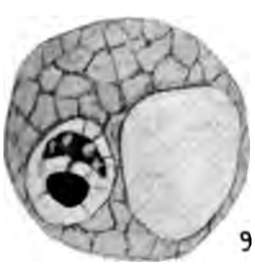

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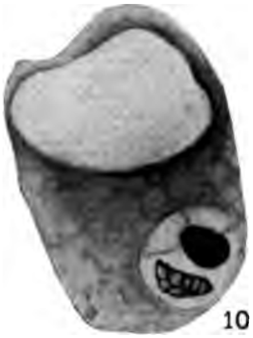

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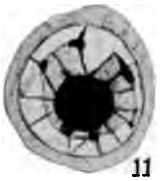

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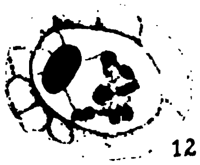

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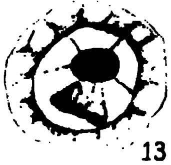

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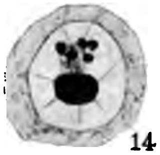

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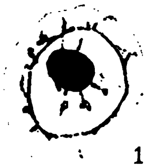

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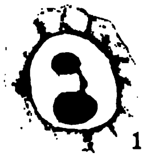

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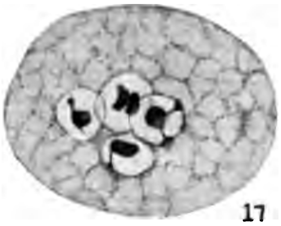

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PLATE IX.

to make a careful study of both the motile and encysted forms, with especial reference to the structure of the nucleus. With this background we hope to be able to correlate better the findings of different investigators, and to discuss the nomenclature of the species. The most interesting thing in the present work is that, although our findings support the contentions of Dobell (1919), we have been able to duplicate the findings of other investigators by using different microscopical methods.

HISTORICAL REVIEW.

As stated above, these cysts were first observed in human faeces by Wenyon (1915) who described them as " spherical bodies " with an " iodophilic " inclusion. A year later the same author redescribed them under the name of " iodine cysts " or " I. cysts." In his first paper he suggested that they were probably vegetable in nature, and in the second paper he believed that he had seen them germinate when kept in faeces. In a still later paper Wenyon and O'Connor (1917) state that besides the typical " iodine cysts " there are sometimes present larger spherical bodies which appear homogenous, with a central vacuole, which, they believed, probably " represent large I-cysts which are devoid of iodophilic bodies or structures of an allied nature."

Kuenen and Swellengrebel (1917) found the " iodine cysts " and an active amoeba, which very probably was the active amoeboid form of the cysts. They provisionally named this active amoeba, " Pseudolimax."

Matthews (1918) again described and figured " iodine cysts." This investigator followed Wenyon's suggestion that they were probably of a vegetable nature.

Flu (1918), in the same year, came to the conclusion that these cysts were, in reality, degenerate cysts of *E. tetragena* (*Endamoeba histolytica*).

After reconsidering the whole matter, Brug (1919) came to the conclusion that the " iodine cysts " are the encysted stage of an amoeba described by Prowazek (1911) under the name, *Entamoeba williamsi.* He was also of the opinion that this amoeba should be placed, with *E. nana,* in the genus *Endolimax* Kuenen and Swellengrebel, 1917. In this paper Brug gives a description of the nucleus of the motile amoeba as well as the cysts. The noteworthy feature of

fixation and available stains, to demonstrate in the nucleus of *C. lafleuri,* the eosinophilic granules which Dobell describes for the amoeba of the " iodine cysts." They even suggest that, with this exception, there is enough in common with their description of *C. lafleuri* and Dobell's description of *Iodamoeba* to make it desirable to reinvestigate Dobell's *Iodamoeba* to see whether or not it should not be considered *C. lafleuri.* It is, however, a little difficult to see their grounds for this suggestion, as the range of size of the two species is not at all similar—*Councilmania* being a very large amoeba and *Iodamoeba* being a very small form.

Brug (1921) has also returned to the study of the amoeba of the " iodine cysts." His description of the amoeba and its cysts is essentially the same as that given in his earlier paper. He also still feels that the amoeba should be called *Endolimax williamsi* Prowazek, 1911. In regard to the nomenclature he has one very interesting new fact to offer, in that he has been able to examine Prowazek's original slides of " Entamoeba williamsi." He finds that these slides contain both *E. coli* and the amoeba of the " iodine cysts." He feels, therefore, that although *E. bütschlii* Prowazek, 1912, is entirely the amoeba of the " iodine cysts," *E. williamsi* Prowazek, 1911, is in part the same species, and that the latter name should have precedence over the former.

Nöller (1921) has been able not only to examine Prowazek's original slides of *E. williamsi,* but also of *E. bütschlii.* This examination supports the contentions of Brug. Nöller feels, however, that since Prowazek's description of *bütschlii* is based solely on the amoeba of the " iodine cysts," whereas the description of *williamsi* is based only in part on this amoeba, the sensible thing is to accept the former name. He therefore uses the name, *Iodamoeba bütschlii* Prowazek, 1912, emend. Dobell, 1919.

Besides the work cited above, Rodenhuis (1919) has proposed the name *Endolimax pileonucleatus* for the amoeba in question. The author's grounds for proposing this name are not clear. One thing of interest in this paper is that the author has found and described what he believes to be several stages in the division of the nucleus. In the paper in which Chauchemez (1921) describes the finding of " iodine cysts " in the faeces of pigs, he notes that Brumpt (personal communication) accepts Dobell's genus *Iodamoeba,* but because he does not believe that the amoeba can be identified with any previously described species, he (Brumpt) proposes the name *Iodamoeba wenyoni.*

MATERIAL AND METHODS.

While a number of stools containing " iodine cysts " have come to our notice during the last two years, practically all of the present work was carried out on specimens obtained from two individuals. These individuals were selected because we could obtain daily samples of faeces from them, and no objection was raised whenever we wished to administer a purgative in order to obtain the motile amoebae. They are sisters, aged three and five years, who were born in Baltimore and have not left the state of Maryland during their life time. We have designated them Cases 1 and 2 respectively. As far as we can find out, they are very healthy children who have no history of intestinal disorder. They show a rather heavy multiple infection of intestinal protozoa, having, besides the amoeba of the "iodine cysts," *Endamoeba coli, Trichomonas hominis,* and *Giardia intestinalis.* Besides these entozoa with which both cases are infected, Case 1 is infected with *Dientamoeba fragilis.* Neither case is infected with *Endolimax nana.*

In addition to studying the organisms in the living condition, they were fixed and stained in various solutions. Most of our material was fixed in Schaudinn's sublimate-alcohol solution, to which were added varying amounts of acetic acid, and then stained either with Heidenhain's iron haematoxylin, followed with eosin, or with Mann's methylblue-eosin stain, as modified by Dobell. A large amount of material was, however, fixed and stained in other ways in order to study it from a comparative standpoint. Among these latter fixatives may be mentioned: Carney's acetic acid-alcohol-chloroform mixture, Fleming's chromo-acetic-osmic mixture (strong formula), Bouin's picro-formol mixture, and chromo-aceto-formalin mixture. The other stains that were used were Delafield's haematoxylin and Dobell's alcoholic iron-haematein.

It is a pleasure to ackowledge our indebtedness to our laboratory assistant, Mr. Conrad Bauer, for finding the cases described above, and for obtaining faecal specimens from them daily over a period of several months. We are also indebted to Miss Ethel Norris for the drawings which appear in this paper.

THE MOTILE AMOEBA.

In the present paper we will limit ourselves to a detailed description of the nucleus of the present form. There are, however, certain

The chief differences between these four types of infection can be explained by assuming a peculiar type of constitution either in the given species of trypanosome or in the host. The peculiar life history of *T. cruzi* can probably be best explained on the first assumption. This is a tissue—rather than a blood-parasite. In the other cases, on the other hand, the chief underlying cause, which determines whether a given infection is of a continuous or a relapsing type, seems to be best explained by assuming that in certain hosts there is some condition (temporary or permanent) which is adverse to the trypanosomes, while in others there is little or no hindrance to the life and reproduction of the parasites. That this is the true explanation of these differences is supported by the work of Pearce and Brown (1918), who have shown that the same species of pathogenic trypanosome exhibits different, but characteristic life histories in different hosts. Even the peculiar type of infection shown by *T. lewisi* in the rat is modified into, what appears to be, a continuous type of lethal infection when this organism is grown in mice (Roudsky, 1911).

In the present work we shall consider any of these conditions which are adverse to the life of the parasite as *resistance*. Resistance in this sense may hence include both a direct resistance, the result of a specific activity on the part of the host, as well as an indirect resistance, resulting from the exhaustion of some essential foodstuffs.

With the exception of *T. cruzi*, with which we will not deal in this paper, a consideration of the facts given above seems to indicate that the highest grade of resistance is encountered in the case of a nonpathogenic trypanosome, such as *T. lewisi* in the rat. Here all reproduction of the parasites ceases 22 to 32 days after the beginning of the infection. The parasites then exist in the blood for 7 to 100 days and finally disappear altogether. Likewise the animal is immune to another infection with *T. lewisi*. The next grade of resistance is that which causes temporary decreases or even disappearances of the parasites from the peripheral blood. This type of resistance is observable in the relapsing infections, such as *T. brucei* in the guinea pig. Here the resistance is often very marked, but it is rarely ever complete, as the host almost invariably succumbs to the infection. In the continuous types of infection, such as *T. brucei* in the rat, there is very little, if any, resistance encountered by the parasites. Apparently the organisms, once they appear in the blood, continue to multiply until the host succumbs.

Any resistance to a trypanosome infection may do one or both of two things: (1) it may retard the rate of reproduction of the parasites, or (2) it may destroy them after they are formed. A fairly accurate picture of the sum total of the resistance of the host may be obtained by making daily determinations of the number of trypanosomes per cmm. of blood. If, for example, the parasites either remain constant or decrease in number for a certain period, we know some type of resistance is operative, but we do not know whether it is producing its effect by retarding the rate of reproduction of the parasites or destroying them after they are formed. That this is true is shown by a consideration of the following equation which gives the factors determining the number of parasites per cmm. of blood.

Number of parasites per cmm. of blood = Number produced by reproduction of parasites — Number destroyed, in consequence of the resistance.*

A further consideration of this equation shows that if we can supplement the enumerative studies with a measure of either the rate of reproduction of the parasites or with a measure of the rate of their destruction, we can differentiate between the two types of the resistance.

In the present work we have attempted to differentiate between the two different types of resistance by supplementing the daily counts with a measure of the rate of reproduction of the trypanosomes. It would probably be impossible to get an absolute measure of this rate unless we could preserve all of the progeny which are produced, and this is obviously not possible, if some are being destroyed. Our measure of this rate is, therefore, a comparative one, and is based on the difference in the variability in size in the individuals of a sample taken, on the one hand, from a population of organisms undergoing rapid multiplication, and, on the other hand, from a population of organisms undergoing little or no multiplication. It is a matter of common observation that a sample from a population of the first type, which necessarily consists of adults and many young forms in different stages of growth, will exhibit a higher variability in size than a sample from one of the second type, which consists largely of adult forms which are more or less uniform in size. Furthermore, it is very important to note that the greater the rate of reproduction with its production of young forms, the greater the variability in the size of individuals from samples of that population. In other words, it follows from these rather self-evident facts that a measure of the variability of size in the individuals of different samples will give us a comparative measure of the rate of reproduction (or production of young forms) in the different populations from which these samples are taken. Although such a method is an indirect one, its great advantage lies in the fact that it is independent of the number of individuals that are killed off or removed, as long as enough individuals are left to enable us to get a sample of suitable size for statistical analysis, and as long as the destructive agent does not select individuals of a given age.

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* The objection might be raised that a disappearance of the trypanosomes from the blood does not necessarily mean that the organisms are destroyed, because they may have simply migrated into the tissues. While trypanosomes undoubtedly do occur in the tissues, there is no evidence that they occur in greater quantities there, when they begin to decrease in the blood. On the other hand, Levaditi and McIntosh (1910) have found trypanolytic antibodies, produced after the crises in the relapsing types of infection, which indicates strongly that the trypanosomes are actually destroyed.

There are two methods of measuring the amount of variability in size of the individuals in a given sample. The first method is that of obtaining the standard deviation, which is a measure of the amount the individuals deviate from the mean or average, expressed in concrete units, such as microns. The second method is that of obtaining the coefficient of variation, which measures the deviation in percentages of the mean. The second constant is the more valuable from our standpoint, because it is a relative measure and allows us to compare the variability of two samples regardless of differences in their mean size.

The method of measuring the rate of reproduction by ascertaining the coefficient of variation in size of random samples is especially applicable in the case of the trypanosomes. In our work we have used the coefficient of variation for total length. The parasites reproduce almost exclusively by binary fission. During this process the organisms grow somewhat longer in size and then divide by an oblique longitudinal fission, producing one fairly long and one very short form. It is therefore evident that the higher the rate of reproduction the greater the number of short forms that will be produced, and in consequence the higher will be the coefficient of variation for total length.

Just how great a difference in variability is found between a sample taken from a population in which rapid reproduction is taking place and one in which there is little or no reproduction can be easily visualized by examining Fig. 1. This figure shows the lengths of two sets of *T. lewisi* from rat 105. Set *A* contains ten trypanosomes, drawn at random, and arranged according to the order of their decreasing lengths, from a slide made the first day after trypanosomes appeared in the blood, at which time they are known to be multiplying actively. Set *B* is a similar set of ten trypanosomes drawn from a slide made on the twenty-eighth day of the infection, at which time all multiplication is known to have ceased. The great variability in size in those specimens (set *A*) which are undergoing rapid multiplication, and those (set *B*) which are not, is evident. While the coefficient of variation was not worked out for these actual samples, it was compiled for larger samples (100 individuals), taken at the same time. The coefficient of variation for a sample of 100 individuals from the same slides from which set *A* was drawn was 25.32 per cent. and that for a similar sample from the slides from which set *B* was drawn was 2.80 per cent.

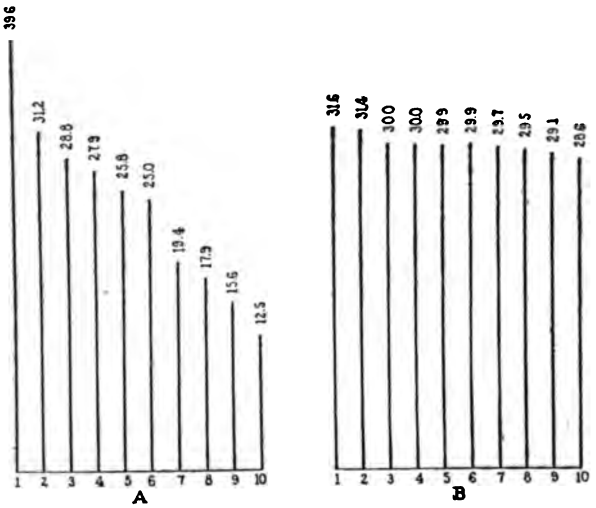

FIG. 1. The lengths of two samples of trypanosomes from rat 105 plotted according to scale and arranged in order of their decreasing lengths. The actual length in microns is also shown. *A*, ten specimens taken at random from the fourth day of the infection; *B*, ten specimens taken at random from the twenty-eighth day. Note the great variability in length of those specimens (*A*) known to be undergoing rapid reproduction, and the uniformity in length of those (*B*) which are known not to be reproducing.

The rationale of the use of the coefficient of variation for total length as a measure of the rate of reproduction is, we believe, evident from this discussion. Since the conclusions reached in the following experiments rest on the assumption that this is a valid method, we feel that it is necessary to completely justify its use. It has to be shown, for example, that the method actually works for trypanosomes, and that no errors are introduced by the possibility mentioned above, namely, the selective killing of trypanosomes of a given age and size, but so much of the proof of the validity of the method rests on the data given in the following experiments that we will present these first. We will, therefore, tentatively assume that the method is valid, and will defer a more complete discussion of it until after the data have been presented.

A. The problem. A brief recapitulation of the problems and methods of attack which are dealt with in this paper may be given as follows: The resistance which a host offers toward a trypanosome infection may either retard the rate of reproduction of the parasites or may actually destroy them after they are formed. These two types of resistance can be differentiated: (1) by making daily counts of the number of trypanosomes per cmm. of blood, and (2) by ascertaining the comparative rate of reproduction of the trypanosomes as is indicated by their coefficient of variation for size. These determinations make it possible to ascertain the relative value of the two types of resistance, because the number of parasites per cmm. of blood at a given time = the number produced by the reproduction of the parasites — the number destroyed in consequence of the resistance, and, if we can ascertain any two of these factors, the third can be estimated. The validity of this method of attack has already been discussed, and will be taken up in greater detail on page 305.

B. Material. The different strains of trypanosomes used in this work have been obtained from several sources. Our strain of *T. lewisi* was obtained from wild Norway rats living on one of the Baltimore city dumps. *T. equiperdum* was furnished to us by Dr. John S. Buckley of the Bureau of Animal Industry. Our *T. equinum* is from Dr. F. G. Novy's strain, and was secured through the kindness of Dr. Paul A. Lewis of the University of Pennsylvania. Both *T. brucei* and *T. rhodesiense* were received from Dr. Wade H. Brown of the Rockefeller Institute. Dr. Brown informs us that there is some uncertainty as to whether the strain which we have designated *T. rhodesiense* is in reality this species. According to him the strain came originally from a patient in East Africa supposedly infected with *T. rhodesicnse*. After it was kept at the Rockefeller Institute for upwards of a year it showed certain morphological changes which have raised some question in regard to its identity. From the standpoint of the present work, however, this is not of very great importance. It is a pleasure to acknowledge our great indebtedness to these investigators, without whose courtesy we would have been unable to carry out the present work. Mention should also be made of the valuable assistance received from our laboratory assistant, Mr. Conrad Bauer.

C. Measurements of size. As our measure of the rate of reproduction of the trypanosomes is based on determinations of the coefficient of variation in size, of samples taken from different infections, it is necessary to select some distance on the body of the trypanosomes, as a measure of size, whose magnitude is different in young and adult forms, and whose determination can be easily and accurately made. A consideration of the structure of a trypanosome suggests several such measurements. The shape of the body in *T. lewisi*, for example, can be seen by an examination of Fig. 2. The chief organelles are a more or less centrally placed nucleus, a blepharoplast from which a flagellum arises, and a parabasal body which is generally so closely associated with the blepharoplast as to obscure it. The proximal portion of the flagellum which extends along the side of the body is united with the latter by the so-called undulating membrane. In the pathogenic species these structures are slightly modified in that the total length of the body is less, the width is greater, the distance from the posterior end to the parabasal body is less, and the parabasal body is smaller.

In our work on *T. lewisi* and *T. equiperdum*, we calculated the coefficient of variation for all of the six distances shown in Fig. 2, viz., (1) posterior end *to* parabasal body, (2) parabasal body *to* nucleus, (3) nucleus *to* anterior end, (4) anterior end *to* end of flagellum, (5) total length, which includes these four measurements, and (6) width, which was taken midway between the parabasal body and the nucleus. It can readily be seen that all of these distances are smaller in specimens which have just divided (young forms) than in specimens just before division ("adult" forms). It was found, therefore, that a study of the coefficient of variation of any of these distances indicated the changes in the rate of reproduction. After a careful consideration of the various measurements, however, we decided to use only total length. This distance is undoubtedly the best single measurement of size for our purpose because: (1) it is the longest distance, and hence, personal errors in measuring it are proportionately less than for the shorter distances, and (2) some of the distances between organelles are intrinsically more variable due to the shifting back and forth of the organelles (see Taliaferro, 1922).

D. Computations. As we have pointed out above, we can measure the degree in variation of size in samples by obtaining either the standard deviation or the coefficient of variation. The latter constant is the more valuable from our standpoint, because it expresses the standard deviation as a percentage of the mean, and is, therefore, available in comparing the degree of variation between samples possessing different mean values. It can be expressed by the following formula:

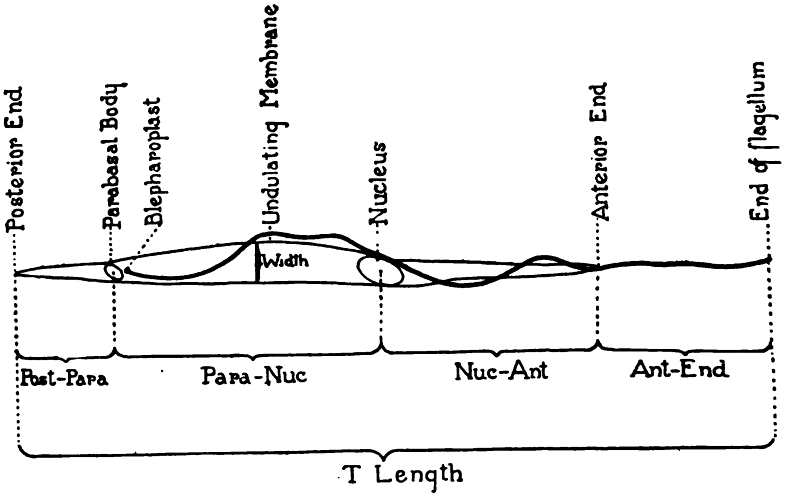

FIG. 2. Diagram of *Trypanosoma lewisi*, projected along a straight line, showing the parts of the organism and the distances used in determining variability in size. *Total length* was found to be the most suitable of these distances, and is the one used in this study. The designation of the parts of the body run vertically, while those of the distances run horizontally.

$$\text{C. V.} = \frac{100\sigma}{M},$$

in which σ is the standard deviation and M the mean. The mean is the average value, and is obtained by dividing the sum of the various measurements by the number of individuals ($\Sigma x/n$). In obtaining the standard deviation we have used the following formula:

$$\sigma = \sqrt{\frac{\sum x^2}{n} - M^2},$$

in which M is the mean, n is the number of individuals, and x denotes the magnitude of the measurements, *not their deviations.*

It is impossible to form any idea of the statistical validity of the constants obtained from these formulae unless the probable error is known. For this reason the probable error for most of the constants has been worked out with the aid of Pearson's Table for Statisticians and Biometricians. With the exception of the data for *T. lewisi,* it seemed unnecessary to give the probable errors for any more than the constants of one day of each infection, because they vary very little in magnitude from day to day, and are all computed from samples of the same number (i.e., 100 individuals). In the case of *T. lewisi* the probable errors are given for the first and last days of the infection. Besides these probable errors Table 1 is appended which gives the probable errors of the coefficient of variation for the integral numbers 2 to 19 per cent., where *n* equals 100. This table enables one to ascertain approximately the probable error of all of the coefficients of variation the probable errors of which are not given in the tables.

TABLE 1.

The probable errors (P. E.) for the coefficient of variation (C. V.) from 2 to 19 per cent. where n equals 100.

C. V.	P. E.	C. V.	P. E.	C. V.	P. E.
2.00	.09	8.00	.38	14.00	.68
3.00	.14	9.00	.43	15.00	.73
4.00	.19	10.00	.48	16.00	.78
5.00	.23	11.00	.53	17.00	.83
6.00	.28	12.00	.58	18.00	.88
7.00	.33	13.00	.63	19.00	.93

E. Enumeration of trypanosomes. Several methods have been devised for counting blood-inhabiting protozoa, such as trypanosomes. One of these methods is that of Kolmer (1915), who dilutes the blood with a fluid which hemolyzes the red cells, and then counts the trypanosomes with the ordinary blood counting methods. Ross and Thomson (1911), in their enumerative studies on trypanasomes, made a thick smear of a known quantity of blood, and then, after plasmolyzing the red cells, counted the trypanosomes on the slide. In our work we made red blood cell counts for our various experimental animals, and then made thin, even blood smears from them. After these blood smears were stained, the red blood cells and trypanosomes in a large number of microscopic fields were counted and the ratio between them calculated. Knowing the number of red cells per cmm. and the ratio of trypanosomes to the red cells, it was an easy matter to compute the number of trypanosomes per cmm. of blood. In a number of cases we checked our method with that of Kolmer and the two gave very closely comparable results.

F. Microscopical technique. In staining trypanosomes, preparatory to measuring them and computing their coefficient of variation for total length, it is necessary to select a technique which will distort the organisms as little as possible, because such distortion probaby would affect their variability in size. In a previous paper on size and variability in *T. lewisi* (Taliaferro, 1922) we came to the conclusion, in agreement with Minchin (1909), that the truest size relationships in this trypanosome can be obtained by fixing in osmic vapor, followed by one of the Romanowsky stains, and drying the slides in an early stage of the process. These dried specimens give the truest size relationships although they are not as suitable for cytological work as the "never-dried" or wet preparations. In the present study, this technique was followed in the case of *T. lewisi*. It is given in detail in the foregoing paper (Taliaferro, 1922). The technical difficulties of this process were so great, however, that for the pathogenic trypanosomes, studied in this paper, we used the ordinary clinical technique of making thin blood smears, drying them, without previous osmic acid vapor fixation, and staining in Giemsa's blood stain. We have already shown, in the above paper, that the omission of the osmic vapor fixation causes a slight shrinkage in size, but it does not produce an appreciable effect on the coefficient of variation, and hence, is perfectly well suited for our purposes, because we are not interested in size *per se*, but in variability in size.

After the slides were stained, they were examined and the midline of a hundred trypanosomes, from each, drawn at a magnification of 3,000, with the aid of a camera lucida. These midlines were then stepped off with a pair of dividers, the points of which were placed 3 mm. apart. The number of steps obtained for each line represented the length of the trypanosome in microns. It is to be emphasized that in drawing specimens from a given slide, every specimen was drawn just as it was encountered, until the requisite number was obtained. In other words, there was no selection of the individuals, but the 100 trypanosomes represented, as near as possible, a true random sample.

G. Experimental procedure. In presenting the various experimental trypanosomiases which follow, we have classified them according to the host rather than the species of trypanosome. This was thought advisable because, with a few exceptions, the species of host, rather than the species of trypanosome, seems to be the factor which determines the type of the resulting infection. Since our object in all these experiments was the same, viz., to differentiate that type of resistance which retards the rate of reproduction of the trypanosomes, and that type which destroys them after they are formed, the experimental procedure in all was the same. The host animal was inoculated intraperitoneally and its blood examined until trypanosomes appeared in the peripheral circulation. As soon as these could be demonstrated, blood smears were made at frequent intervals throughout the course of the infection and were stained, as indicated under *Microscopical Technique.* At the same time the slides were made, the red blood cells were generally counted with an ordinary hemocytometer. The slides were first examined and 100 trypanosomes drawn and measured. From these 100 measurements the coefficient of variation for total length for that day was calculated. The slides were then examined again and the ratio between red cells and trypanosomes ascertained, which, in conjunction with the red blood cell count, enabled us to estimate the number of trypanosomes per cmm. of blood.

THE RAT AS A HOST OF TRYPANOSOME INFECTIONS.

A. A non-pathogenic infection. We will consider a non-pathogenic trypanosome first, because the very fact that it is not pathogenic to its host indicates that the host builds up a high grade of resistance against it. Elsewhere (Taliaferro, 1921 *a* and 1922), we have described the infection with *T. lewisi* in the rat in great detail and it will suffice to merely give a summary of the facts here. The course of the infection can be divided as follows:*

1. Multiplicative stage (twenty-two to thirty-two days).
    - *a.* Incubation period during which no organisms are in the blood (four to eight days).
    - *b.* Multiplicative period in the blood (eighteen to twenty-four days).
2. Stage of "adult" infection (seven to one hundred days).

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* In one of these papers (Taliaferro, 1921 *a*), we gave the different stages in the infection in terms of the *blood infection*, which starts only after the organisms appear in the blood. We now give these stages in terms of the entire infection which starts at the time of injection. This accounts for the apparent difference in terminology given here and in that paper.

During the multiplicative stage the organisms are undergoing more or less rapid reproduction, or at least growth, whereas during the "adult" stage of the infection they exist in the blood without any signs of division or growth. At the end of the "adult" stage, they disappear from the blood, and the rat is immune to another infection with this form.

T. lewisi: rat 105.

Protocol 105. Culture rat infected with *T. lewisi.* Injected intraperitoneally with 1 c.c. of a 1–100 dilution of blood from rat 116 on May 1, 1920.[*] Organisms first demonstrated in the blood on May 5, 1920. Trypanosomes disappeared from the blood on June 8, 1920, and were never found after this date, although repeated blood examinations were made. Trypanosomes were measured and the coefficient of variation for total length was calculated on 12 days, as is shown in Fig. 3 and Table 2. Estimations of the number of organisms per cmm. of blood were made on 13 days, as is shown in the same tables.

Incubation period was 4 days.

Duration of infection was 35 days.

TABLE 2.

Mean, standard deviation, and coefficient of variation for total length on 12 days and the number of trypanosomes per cmm. of blood on 13 days during the course of the infection with T. lewisi in rat 105.

Each statistical constant is based on the measurement of 100 individuals, 1200 trypanosomes having been measured during this infection. See Fig. 3 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	4 days.	6 days.	8 days.	10 days.	12 days.	14 days.
Mean length	24.785±.423	27.668	30.108	30.519	......	29.904
Standard deviation	6.277±.299	5.226	4.159	1.206		1.453
Coefficient of variation	**25.32±1.28**	**18.88**	**13.81**	**3.95**		**4.85**
Number of trypanosomes per cmm. of blood	**41,000**	**277,000**	**301,000**	**338,000**	**145,000**	**76,000**

Interval after injection.	18 days	22 days	26 days	28 days	30 days	33 days	35 days.
Mean length	30.481	31.412	31.723	31.251	31.490	31.815	30.654±.069
Standard deviation	1.788	.977	1.250	.875	.952	.884	1.036±.049
Coefficient of variation	**5.86**	**3.11**	**3.94**	**2.80**	**3.02**	**2.78**	**3.38±.16**
Number of trypanosomes per cmm. of blood	**67,000**	**69,000**	**62,000**	**57,000**	**56,000**	**52,000**	**45,000**

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[*] All dilutions of blood were made with 0.9 per cent saline.

motile amoeba and, therefore, if it can be shown that the motile stage of *williamsi* is different from *E. coli* we will have to retain the name *williamsi* for this new species, and it will not be permissible to consider it a synonym of *E. coli*. Not only have Nöller (1921) and Brug (1921) shown that Prowazek's original slides contained both *E. coli* and the amoeba of the "iodine cysts," but an examination of Prowazek's description of *E. williamsi* shows that the motile stages of this form (Figs. 1 and 2) are those of the "iodine cysts." A further fact of paramount importance is that Brug (1921) clearly designated Prowazek's original slides as the type material of the species. The revision of Prowazek's *williamsi* by Hartmann (1913), the designation of Prowazek's original material as the type material of *williamsi* by Brug (1921), and the identification of this material with the amoeba of the "iodine cysts" by Brug (1921) and Nöller (1921), make it quite clear to us that under the International Rules of Nomenclature (Articles 29, 30, and 31) *williamsi* is the valid name of the species in question.

To sum up, then, we feel that the name of the amoeba of the "iodine cysts" should be *Iodamoeba williamsi* Prowazek 1911.

CONCLUSIONS.

1. In the nucleus of the motile amoeba of the "iodine cysts" there is a well-defined nuclear membrane, a large central karyosome, a single layer of peripheral granules ("peripheral chromatin") which surrounds the karyosome, and a few fine linin strands which run from the karyosome through the layer of peripheral granules to the nuclear membrane. The peripheral granules show a different staining reaction from the karyosome.

2. The nucleus of the cysts contains the same structures as that of the motile amoebae. The karyosome, however, becomes eccentrically placed, and the peripheral granules, which vary greatly in number among different nuclei, become localized on one side of the karyosome, between it and the nuclear membrane.

3. With the exception of the linin strands, our description agrees with that of Dobell. The crescent-shaped mass which is described by Brug and Nöller in the nuclei of the cysts, is composed of the granules noted above. In cysts which are stained with iron haematoxylin and decolorized to too great an extent we have found many pictures which remind one very much of *E. nana,* and may explain the findings of Kofoid, Kornhauser and Swezy.

which shows graphically the difference in variability between the organisms on the fourth and twenty-eighth days of this infection. We do not feel that there is any doubt that these changes are due to changes in the rate of reproduction of the trypanosomes. When they first appear in the blood, a microscopical examination of the slides shows that they are undergoing rapid multiplication. Actual division probably ceases by the tenth day. The slight decline noticed from the tenth to the twenty-eighth day is probably the result of the growth of a few young forms still in the blood. From the 28th day to the end of the infection, there is no morphological evidence either of division or growth.

The question might be raised, if the coefficient of variation gives a comparative measure of the rate of reproduction, how can we say there is no reproduction taking place when the coefficient of variation is between 3 and 4 per cent. This cannot be answered with absolute certainty, but we know that a population of adults undergoing no reproduction still exhibits a certain amount of variability in size, although the degree in variation is far less than for the same population undergoing rapid reproduction. These conclusions concerning the reproduction of the trypanosomes, which are drawn from a study of the changes in the coefficient of variation for total length, are supported by a microscopical study of the slides and are discussed in detail on page 306.

From the standpoint of the host's resistance to the infection, these results indicate that the host quickly built up a resistance which retarded the rate of reproduction more and more, so that all actual division of the parasites was inhibited by the tenth day of the infection. Let us see how these facts tally with the changes in the number of parasites per cmm. of blood. During the period extending from the first day after the trypanosomes appeared in the blood until the tenth day of the infection the parasites rapidly increased in numbers, reaching the high number of 338,000 per cmm. of blood. The rate of increase during this period, however, was not uniform, but was more rapid during the first two days than the last part of the period. In other words, the rate of increase was progressively less during this period, which is to be expected since our study of the coefficient of variation for total length indicated that the rate of reproduction was also progressively less during the same period. From the tenth to the fourteenth day, the parasites suddenly decreased in number (76.000 per cmm. on the fourteenth day). This sudden decrease can not be accounted for on the basis of the cessation of reproductive activity alone, because, had this been the only factor involved, the number of parasites would have remained stationary. We must, therefore, conclude that, at this point, the host has built up a resistance not only toward a retardation of the rate of reproduction, but also toward an actual destruction of the parasites after they are formed. From the fourteenth day until the thirty-fifth day, the number of parasites in the blood remained practically constant with the exception of a slight decrease. Between the thirty-fifth and thirty-sixth days they completely disappeared from the blood and could never be found again although repeated attempts were made to demonstrate their presence.

To sum up the results obtained in this infection with *T. lewisi* in the rat, we find: (1) A resistance was built up which retarded the rate of reproduction of the parasites more and more, and finally inhibited it altogether (by the tenth day). It is true that growth of the organisms may have taken place for some days more, but apparently all actual multiplication was stopped by this time. (2) Between the tenth and fourteenth days, a resistance was developed which killed off a large number of the parasites, i.e., it was directed toward their destruction after they were formed. Finally, (3) a resistance of the second type was again developed by the thirty-fifth day which caused the complete destruction of all the remaining organisms.

In the present paper we will limit our discussion simply to the different *effects* of the host's resistance and not its *nature*. It is possible that these different effects are due to the same fundamental type of resistance. This is notably true of the first and second effects given above. We will call attention to the fact, however, that the agency which finally destroys the trypanosomes at the end of the infection and that which causes the first decline in numbers is probably different. We feel this to be true, because the time which elapses between the two varies to such an extent. In the present infection this interval was about twenty-five days, but it may vary from a week to several months.

Rat 116.

Protocol 116. Culture rat infected with *T. lewisi.* Injected intraperitoneally with a single trypanosome, which was isolated with a Barber pipette, on April 8, 1920. Organisms first demonstrated in the blood on April 16, 1920. Trypanosomes disappeared from the blood on June 26, 1920, and were never found after this date, although repeated blood examinations were made. No clinical symptoms were observed during the course of the infection. Trypanosomes were measured and the coefficient of variation for total length calculated on 6 days, as is shown in Fig. 4 and Table 3. Estimations of the number of organisms per cmm. of blood were made on 5 days as is shown in the same figure and table.

Incubation period was 8 days.

Duration of infection was 79 days.

TABLE 3.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood on 6 days during the infection with T. lewisi in rat 116.

Each statistical constant is based on the measurement of 100 individuals, 600 trypanosomes having been measured during this infection. See Fig. 4 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection	9 days.	25 days.	32 days.	39 days.	49 days.	79 days.
Mean length......	26.029±.470	29.481	30.551	29.271	31.629	31.162±.099
Standard deviation	6.978±.332	2.061	.982	.928	.850	1.469±.070
Coefficient of variation..........	**26.80±1.36**	**6.99**	**3.21**	**3.17**	**2.68**	**4.71±.22**
Number of trypanosomes per cmm. of blood........	....	**504,000**	**164,000**	**68,000**	**43,000**	**12,000**

Fewer determinations were made during this infection than in the previous one. An examination of Fig. 4 and Table 3 shows, however, that the results obtained support the conclusions reached in the pre-

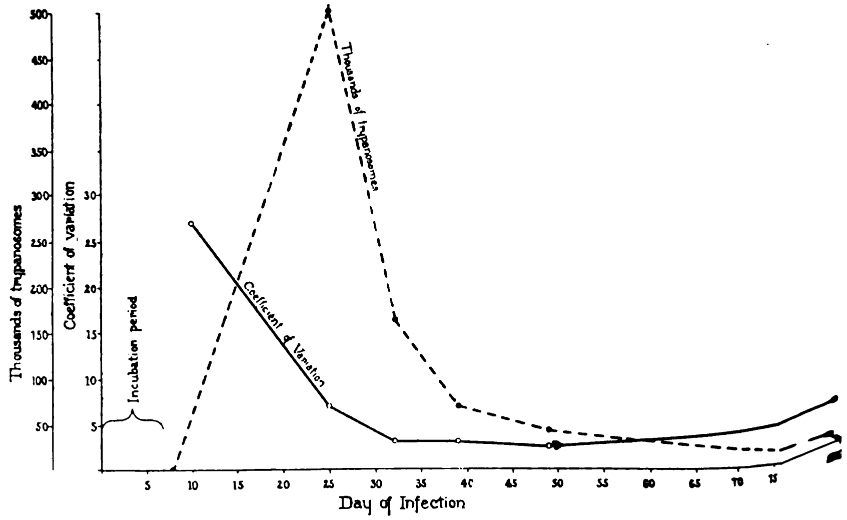
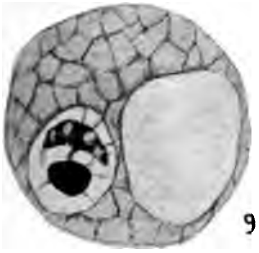


FIG. 4. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. lewisi* in rat 116.



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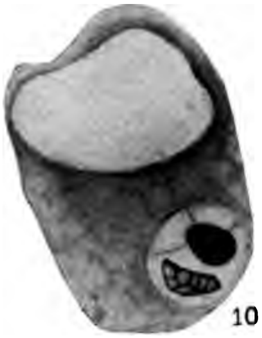

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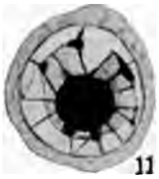

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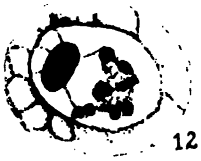

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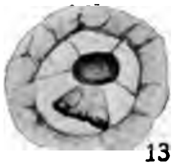

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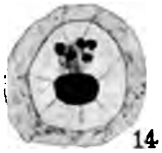

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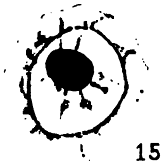

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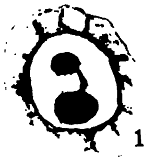

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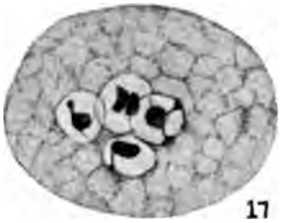

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PLATE IX.

ity of infections with pathogenic trypanosomes in the rat are of the continuous type. From the standpoint of the resistance built up by the host, these infections present just the opposite conditions to those found in a non-pathogenic trypanosome such as *T. lewisi.* In the following experiments we have studied *T. equiperdum* and *T. rhodesiense,* both of which show this type of infection in the rat.

T. equiperdum: rat 619.

Protocol 619. Culture rat infected with *T. equiperdum.* Injected intraperitoneally with 1 c.c. of a 1–100 dilution of blood from a guinea pig which had a heavy infection, on February 17, 1921, at 10:00 A.M. Organisms first demonstrated in the blood on February 19, 1921, at 9:00 A.M. Rat died on February 23, 1921, at 8:00 A.M., the last slides being made during terminal convulsions. Trypanosomes were measured and the coefficient of variation calculated for total length, and estimations of the number of organisms per cmm. of blood were made at 8 periods during the course of the infection, as is shown in Fig. 5 and Table 4.

Incubation period was 1 day 23 hours.

Duration of infection was 5 days 22 hours.

TABLE 4.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 8 intervals during the course of the infection with T. equiperdum in rat 619.

Each statistical constant is based on the measurement of 100 individuals, 800 trypanosomes having been measured during this infection. See Fig. 5 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	2 days 10 hrs.	3 days.	3 days 10 hrs.	3 days 22 hrs.	4 days 12 hrs.	4 days 22 hrs.	5 days 11 hrs.	5 days 22 hrs.
Mean length	23.640±.117	23.812	22.448	24.552	24.880	24.792	23.976	23.574
Standard deviation	1.735±.082	1.788	1.821	1.687	2.280	2.562	2.191	2.579
Coefficient of variation	7.34 ±.35	7.51	8.14	6.87	9.16	10.33	9.13	10.94
Number of trypanosomes per cmm. of blood	7,000	38,000	229,000	194,000	1,333,000	1,710,000	3,692,000	5,102,000

Even a casual glance at Fig. 5 and Table 4, which give the results obtained for this infection, show that the changes both in the coefficient of variation and the number of parasites are entirely different from those of *T. lewisi.* The coefficient of variation was 7.34 per cent. on the first day after the trypanosomes appeared in the blood. This value is considerably lower than that for the same day in the *T. lewisi* infections. Instead of getting lower, however, as it did in the latter infections, it rose until at the time the animal died it was 10.94 per cent. There is no doubt that this increase in the coefficient of variation is significant from the statistical standpoint, because the difference between the constants in these two days is approximately 6 times its probable error. (10.94 ± .52) — (7.34 ± .35) = (3.60 ± .62). The question then arises is this great difference actually due to an increase in the rate of multiplication of the parasite or simply to some experimental error in technique? This question can not be answered until data for more infections of this kind are obtained. The general shape of the graph, showing the changes in the coefficient of variation, indicates that the rise is significant. We are at least justified in concluding that the host does not build up any resistance which is directed toward a retardation of the reproduction of the parasites, as was the case in the non-pathogenic infection with *T. lewisi.* There is, on the contrary, some evidence that the parasite gradually overcomes more and more the natural resistance which the host had at the beginning of the infection.

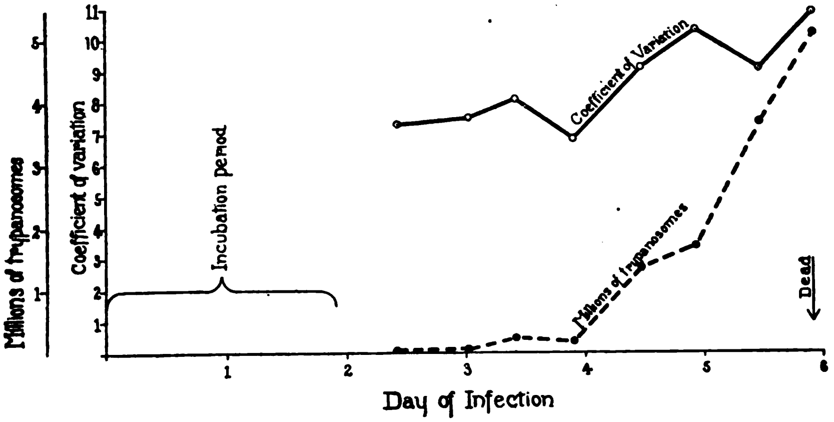

FIG. 5. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. equiperdum* in rat 619.

In an infection of this nature in which there is very little change in the rate of reproduction of the parasites from day to day, we would expect them to increase in the blood according to a curve of geometrical progression. An examination of Table 4 and Fig. 5 shows that there were 7,000 trypanosomes per cmm. of blood at the time of the first determination and that this number rapidly increased until there were 5,102,000 at the time of death. The curve of increase does not exactly follow a curve of geometrical progression. It does, however, strongly suggest a curve of this nature. In this connection it may be noted that the truest approximations to curves of the geometrical progression type were found in the continuous infections in the mouse. In spite of the fact that the trypanosomes did not increase in the blood exactly according to a curve of geometrical progression, we are justified in concluding that the host built up very little, if any, resistance directed toward a destruction of the parasites after they are formed. The results of the preceding paragraph also justify the conclusion that there was no resistance built up which retarded their rate of reproduction. On the contrary there is some evidence that they multiplied faster at the end than at the beginning of the infection.

T. rhodesiense: rat 703.

Protocol 703. Culture rat, infected with *T. rhodesiense.* Injected intraperitoneally with 0.5 c.c. of a 1–200 dilution of blood from a very heavily infected rat, on May 3, 1921. Organisms first demonstrated in the blood on May 4, 1921. Rat was dead by May 11, 1921. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals, and estimations of the number of organisms per cmm. of blood were made 4 times during the course of the infection, on the dates shown in Fig. 6 and Table 5.

Incubation period was 1 day.

Duration of the infection was approximately 6½ days.

The infection in this animal was very similar to the infection of *equiperdum* in rat 619. Both infections were of a very acute type and lasted approximately six days. While the coefficient of variation for total length is, on the average, higher than in rat 619, the changes are of approximately the same character. When the organisms first appeared in the blood the coefficient of variation for total length was 9.20 per cent. This value rose so that by the end of the infection it was as high as 12.36 per cent. Here again we have an increase in the coefficient of variation which is undoubtedly significant from a statistical standpoint $[(12.36 \pm .59) - (9.20 \pm .44) = (3.16 \pm .74)]$.

TABLE 5.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 4 intervals during the infection with T. rhodesiense in rat 703.

Each statistical constant is based on the measurement of 100 individuals, 400 trypanosomes having been measured during this infection. See Fig. 6 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	1 day 20 hrs.	2 days 20 hrs.	3 days 20 hrs.	5 days 20 hrs.
Mean length	25.733±.159	26.418	27.328	26.196
Standard deviation	2.368±.112	2.479	3.284	3.238
Coefficient of variation	9.20 ±.44	9.38	12.01	12.36
Number of trypanosomes per cmm. of blood	31,000	198,000	702,000	3,158,000

indicating that the parasites multiplied more rapidly at the end of the infection than at the beginning. We are, therefore, justified in concluding that not only does the host not build up any resistance which

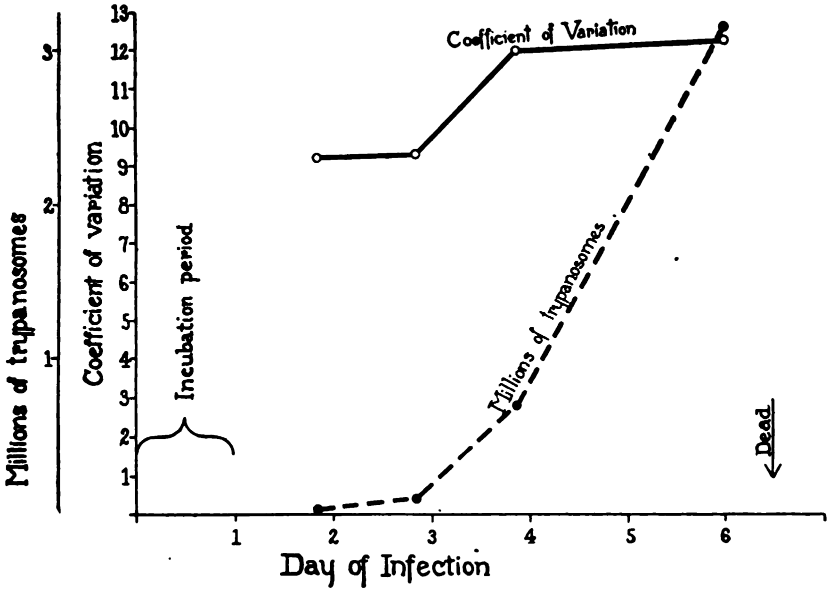

FIG. 6. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. rhodesiense* in rat 703.

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THE RESISTANCE OF DIFFERENT HOSTS TO EXPERIMENTAL TRYPANOSOME INFECTIONS, WITH ESPECIAL REFERENCE TO A NEW METHOD OF MEASURING THIS RESISTANCE.*

By W. H. TALIAFERRO, Ph.D., and L. G. TALIAFERRO, A.B.

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* From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

This is the second of a series of papers dealing with trypanosomes in which we are trying to attack some of the problems dealing with (1) variation and inheritance, and (2) the physiological factors involved in the interaction of host and parasite. The first paper of the series deals primarily with the first subject and is now in press for the Journal of Experimental Zoology (Taliaferro, 1922).

ever, that these fluctuations, as great as they may at first seem, are ely due to experimental errors in technique, and that the true ue of the coefficient of variation was approximately 10 per cent. ughout the course of the infection. This contention is supported

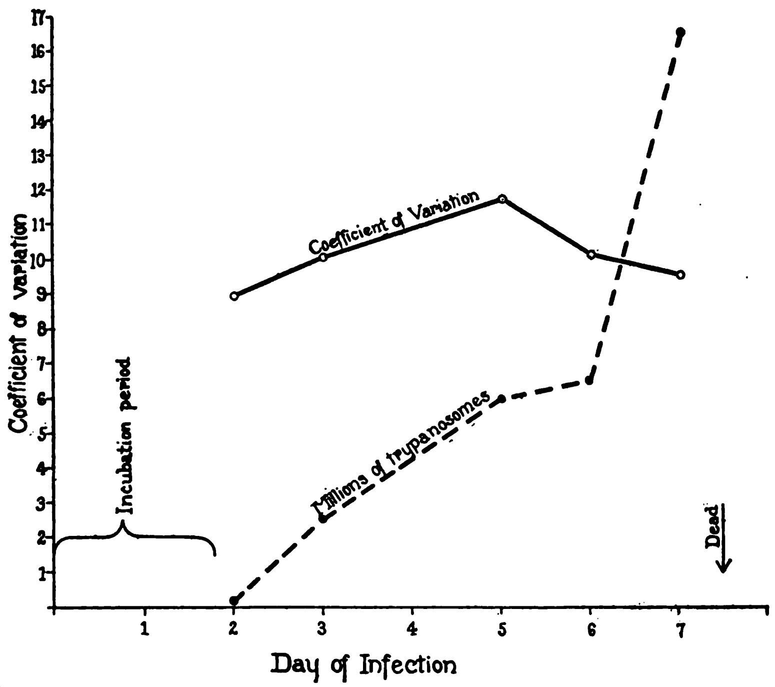

Fig. 7. Graph showing the coefficient of variation for total length (comtive measure of the rate of reproduction) and the number of trypanosomes cmm. of blood, throughout the course of the infection with *T. rhodesiense* in 705.

the fact that the high value reached on the fifth day (11.69 ± .56 cent.) does not show a significant increase from the statistical standat over the preceding (10.09 ± .48 per cent.) or succeeding (10.08 48 per cent.) determination. Its difference from the preceding surement is 1.60 ± .74 which is only two times its probable error, its difference from the succeeding measurement is 1.61 ± .74 which lso only two times its probable error. In any case, there is no lence that the host has built up a resistance of either type to this anosome infection.

Rat 709.

Protocol 709. Culture rat, infected with *T. rhodesiense*. Injected intraperially with 0.5 c.c. of a 1–200 dilution of blood from a very heavily infected checked our method with that of Kolmer and the two gave very closely comparable results.

F. Microscopical technique. In staining trypanosomes, preparatory to measuring them and computing their coefficient of variation for total length, it is necessary to select a technique which will distort the organisms as little as possible, because such distortion probably would affect their variability in size. In a previous paper on size and variability in *T. lewisi* (Taliaferro, 1922) we came to the conclusion, in agreement with Minchin (1909), that the truest size relationships in this trypanosome can be obtained by fixing in osmic vapor, followed by one of the Romanowsky stains, and drying the slides in an early stage of the process. These dried specimens give the truest size relationships although they are not as suitable for cytological work as the "never-dried" or wet preparations. In the present study, this technique was followed in the case of *T. lewisi.* It is given in detail in the foregoing paper (Taliaferro, 1922). The technical difficulties of this process were so great, however, that for the pathogenic trypanosomes, studied in this paper, we used the ordinary clinical technique of making thin blood smears, drying them, without previous osmic acid vapor fixation, and staining in Giemsa's blood stain. We have already shown, in the above paper, that the omission of the osmic vapor fixation causes a slight shrinkage in size, but it does not produce an appreciable effect on the coefficient of variation, and hence is perfectly well suited for our purposes, because we are not interested in size *per se*, but in variability in size.

After the slides were stained, they were examined and the midline of a hundred trypanosomes, from each, drawn at a magnification of 3,000, with the aid of a camera lucida. These midlines were then stepped off with a pair of dividers, the points of which were placed 3 mm. apart. The number of steps obtained for each line represented the length of the trypanosome in microns. It is to be emphasized that in drawing specimens from a given slide, every specimen was drawn just as it was encountered, until the requisite number was obtained. In other words, there was no selection of the individuals, but the 100 trypanosomes represented, as near as possible, a true random sample.

G. Experimental procedure. In presenting the various experimental trypanosomiases which follow, we have classified them according to the host rather than the species of trypanosome. This was thought two things: (1) it may retard the rate of reproduction of the parasites, or (2) it may destroy them after they are formed. A fairly accurate picture of the sum total of the resistance of the host may be obtained by making daily determinations of the number of trypanosomes per cmm. of blood. If, for example, the parasites either remain constant or decrease in number for a certain period, we know some type of resistance is operative, but we do not know whether it is producing its effect by retarding the rate of reproduction of the parasites or destroying them after they are formed. That this is true is shown by a consideration of the following equation which gives the factors determining the number of parasites per cmm. of blood.

Number of parasites per cmm. of blood = Number produced by reproduction of parasites — Number destroyed, in consequence of the resistance.*

A further consideration of this equation shows that if we can supplement the enumerative studies with a measure of either the rate of reproduction of the parasites or with a measure of the rate of their destruction, we can differentiate between the two types of the resistance.

In the present work we have attempted to differentiate between the two different types of resistance by supplementing the daily counts with a measure of the rate of reproduction of the trypanosomes. It would probably be impossible to get an absolute measure of this rate unless we could preserve all of the progeny which are produced, and this is obviously not possible, if some are being destroyed. Our measure of this rate is, therefore, a comparative one, and is based on the difference in the variability in size in the individuals of a sample taken, on the one hand, from a population of organisms undergoing rapid multiplication, and, on the other hand, from a population of organisms undergoing little or no multiplication. It is a matter of common observation that a sample from a population of the first type, which necessarily consists of adults and many young forms in different the organisms which were not killed. It also seems quite probable that this five-day period of resistance was the underlying factor in the increased length of this infection. If this period be removed from our figure and table, our data present a picture almost exactly similar to the ones described for the shorter infections.

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* The objection might be raised that a disappearance of the trypanosomes from the blood does not necessarily mean that the organisms are destroyed, because they may have simply migrated into the tissues. While trypanosomes undoubtedly do occur in the tissues, there is no evidence that they occur in greater quantities there, when they begin to decrease in the blood. On the other hand, Levaditi and McIntosh (1910) have found trypanolytic antibodies, produced after the crises in the relapsing types of infection, which indicates strongly that the trypanosomes are actually destroyed.

C. Pathogenic infection of the relapsing type. Of the trypanosomes which we have studied, only one, namely *T. equinum,* produces a true relapsing infection in the rat. Our general experience with this infection in the rat is closely similar to that of Pearce and Brown (1918). We have, however, worked up the data of only one infection of this type.

T. equinum: rat 729.

Protocol 729. Culture rat, infected with *T. equinum.* Injected intraperitoneally with 1 c.c. of a 1–200 dilution of blood from a lightly infected rat, on October 30, 1921. Organisms first demonstrated in the blood on November 2. Rat was dead by November 9, at 9:00 A.M. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals in each case, and estimations of the number of organisms per cmm. of blood, made 12 times during the course of the infection, on the dates shown in Fig. 9 and Table 8. After the trypanosomes first appeared in the blood they disappeared completely from the peripheral circulation on two occasions during the course of the infection, and could not be found on November 3 at 3:00 P.M., or on November 6, at 9:00 A.M. and 3:00 P.M. These two intervals are indicated in Fig. 9.

Incubation period was 3 days.

Duration of the infection was approximately 10½ days.

TABLE 8.

Mean, standard deviation, and coefficient of variation for total length at 12 intervals and the number of trypanosomes per cmm. of blood at 15 intervals during the course of the infection with T. equinum in rat 729.

Each statistical constant is based on the measurement of 100 individuals, 1,200 trypanosomes having been measured during this infection. See Fig. 9 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	3 days.	3 days 12 hrs.	4 days.	4 days 12 hrs.	5 days.	5 days 12 hrs.	6 days.	6 days 12 hrs.
Mean length. . . .	21.719±.139	21.412	21.677	. . . . . .	21.222	21.694	21.490	23.004
Standard deviation	2.073±.098	1.790	1.803	. . . . . .	2.141	2.004	2.062	2.626
Coefficient of variation	9.54 ±.45	8.36	8.31	. . . . . .	10.09	9.23	9.59	11.41
Number of trypanosomes per cmm. of blood.	4,000	48,000	73,000	000	800	3,000	8,000	9,000

Interval after injection.	7 days.	7 days 12 hrs.	8 days.	8 days 12 hrs.	9 days.	9 days 12 hrs.	10 days.
Mean length....	......	......	22.263	22.373	22.019	22.233	21.654
Standard deviation..........	......	......	2.083	2.220	2.078	2.315	2.346
Coefficient of variation.....	......	......	9.35	9.92	9.44	10.41	10.83
Number of trypanosomes per cmm. of blood.	000	000	483,000	1,171,000	3,436,000	4,869,000	9,707,000

An examination of the data in Fig. 9 and Table 8 indicates that there were no progressive changes in the rate of reproduction of the trypanosomes, as indicated by the coefficient of variation. This remained somewhere in the neighborhood of 10 per cent. throughout the entire infection. The number of the trypanosomes per cmm., however, showed some very marked fluctuations. They appeared in the blood three times during the course of the infection, and disappeared twice. During their first appearance they reached 73,000 per cmm. (the

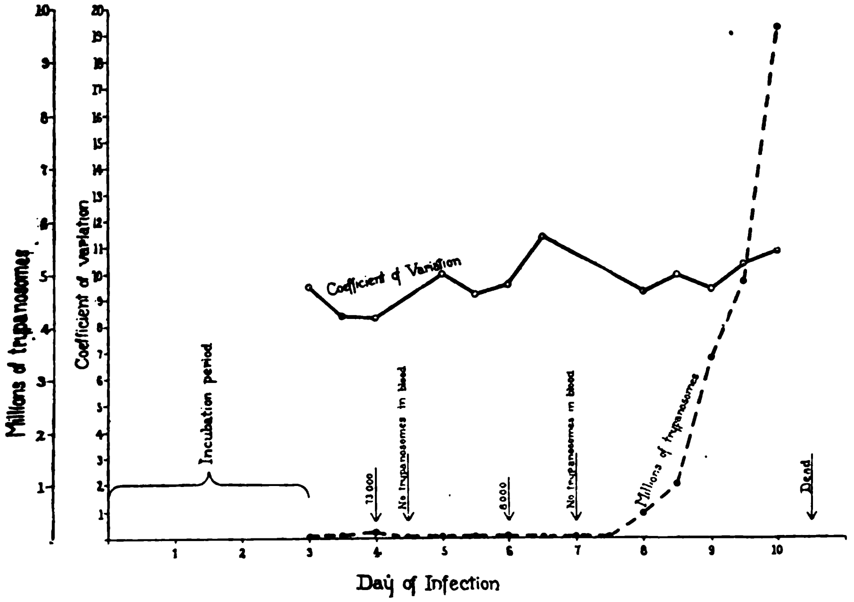

FIG. 9. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. equinum* in rat 729.

highest number), during their second 9,000 per cmm., and during their third 9,707,000 per cmm., culminating in death. These two complete disappearances of the organisms from the blood indicates the development of a resistance which destroys the organisms without any marked influence on their rate of multiplication.

THE MOUSE AS A HOST OF TRYPANOSOME INFECTION.

We have been unable to obtain *T. duttoni* which is a non-pathogenic trypanosome of mice, very similar to *T. lewisi* of the rat. Roudsky (1911), by a special treatment of *T. lewisi,* has been able to grow this organism in the mouse, in which case it develops into a lethal parasite. It would be very interesting to compare the condition of affairs in *T. lewisi,* when it is pathogenic and when it is non-pathogenic, but we have been unable to do this. The following two infections in the mouse are, perhaps, our best examples of infections in which the host builds up no resistance of either type.

A. Pathogenic infection of the continuous type.

T. brucei: mouse 728.

Protocol 728. Culture mouse, infected with *T. brucei.* Injected intraperitoneally with 0.75 c.c. of a 1–200 dilution of blood from a lightly infected guinea pig, on November 28, 1921, at 8:30 A.M. Organisms first demonstrated in the blood on November 29, at 9:00 A.M. Mouse was dead on December 2, at 9:00 A.M. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals in each case, and estimations of the number of organisms per cmm. of blood were made 6 times during the course of the infection, as is shown in Table 9 and Fig. 10.

Incubation period was 1 day.

Duration of the infection was approximately 3¾ days.

TABLE 9.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 6 intervals during the infection with T. brucei in mouse 728.

Each statistical constant is based on the measurement of 100 individuals, 600 trypanosomes having been measured during this infection. See Fig. 10 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	1 day 2 hrs.	1 day 12 hrs.	2 days.	2 days 12 hrs.	3 days.	3 days 12 hrs.
Mean length	22.554±.125	22.336	22.793	22.213	23.005	22.480
Standard deviation	1.865±.088	2.224	2.261	2.247	2.101	2.267
Coefficient of variation	8.27 ±.39	10.05	9.92	10.11	9.13	10.08
Number of trypanosomes per cmm. of blood	900	4,000	10,000	93,000	409,000	4,103,000

is a pleasure to acknowledge our great indebtedness to these investigators, without whose courtesy we would have been unable to carry out the present work. Mention should also be made of the valuable assistance received from our laboratory assistant, Mr. Conrad Bauer.

C. Measurements of size. As our measure of the rate of reproduction of the trypanosomes is based on determinations of the coefficient of variation in size, of samples taken from different infections, it is necessary to select some distance on the body of the trypanosomes, as a measure of size, whose magnitude is different in young and adult forms, and whose determination can be easily and accurately made. A consideration of the structure of a trypanosome suggests several such measurements. The shape of the body in *T. lewisi*, for example, can be seen by an examination of Fig. 2. The chief organelles are a more or less centrally placed nucleus, a blepharoplast from which a flagellum arises, and a parabasal body which is generally so closely associated with the blepharoplast as to obscure it. The proximal portion of the flagellum which extends along the side of the body is united with the latter by the so-called undulating membrane. In the pathogenic species these structures are slightly modified in that the total length of the body is less, the width is greater, the distance from the posterior end to the parabasal body is less, and the parabasal body is smaller.

In our work on *T. lewisi* and *T. equiperdum*, we calculated the coefficient of variation for all of the six distances shown in Fig. 2, viz., (1) posterior end *to* parabasal body, (2) parabasal body *to* nucleus, (3) nucleus *to* anterior end, (4) anterior end *to* end of flagellum, (5) total length, which includes these four measurements, and (6) width, which was taken midway between the parabasal body and the nucleus. It can readily be seen that all of these distances are smaller in specimens which have just divided (young forms) than in specimens just before division (" adult " forms). It was found, therefore, that a study of the coefficient of variation of any of these distances indicated the changes in the rate of reproduction. After a careful consideration of the various measurements, however, we decided to use only total length. This distance is undoubtedly the best single measurement of size for our purpose because: (1) it is the longest distance, and hence, personal errors in measuring it are proportionately less than for the shorter distances, and (2) some of the distances between organelles are intrinsically more variable due to the shifting back and forth of the organelles (see Taliaferro, 1922).

in Fig. 4 and Table 3. Estimations of the number of organisms per cmm. of blood were made on 5 days as is shown in the same figure and table.

Incubation period was 8 days.

Duration of infection was 79 days.

TABLE 3.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood on 6 days during the infection with T. lewisi in rat 116.

Each statistical constant is based on the measurement of 100 individuals, 600 trypanosomes having been measured during this infection. See Fig. 4 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection	9 days.	25 days.	32 days.	39 days.	49 days.	79 days.
Mean length......	26.029±.470	29.481	30.551	29.271	31.629	31.162± 099
Standard deviation	6.978±.332	2.061	.982	.928	.850	1.469± 070
Coefficient of variation..........	**26.80±1.36**	**6.99**	**3.21**	**3.17**	**2.68**	**4.71±.[illegible]2**
Number of trypanosomes per cmm. of blood........	....	**504,000**	**164,000**	**68,000**	**43,000**	**12,00[illegible]**

Fewer determinations were made during this infection than in the previous one. An examination of Fig. 4 and Table 3 shows, however, that the results obtained support the conclusions reached in the pre-

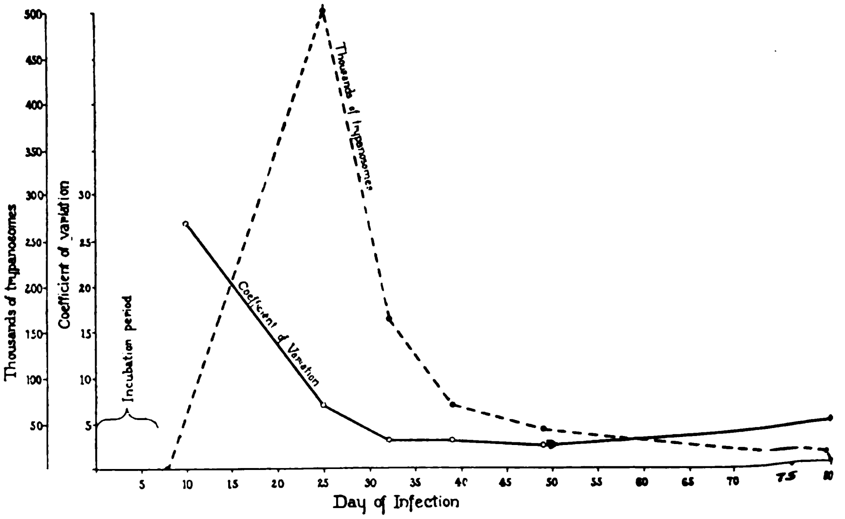

FIG. 4. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. lewisi* in rat 116.

known. For this reason the probable error for most of the constants has been worked out with the aid of Pearson's Table for Statisticians and Biometricians. With the exception of the data for *T. lewisi,* it seemed unnecessary to give the probable errors for any more than the constants of one day of each infection, because they vary very little in magnitude from day to day, and are all computed from samples of the same number (i.e., 100 individuals). In the case of *T. lewisi* the probable errors are given for the first and last days of the infection. Besides these probable errors Table 1 is appended which gives the probable errors of the coefficient of variation for the integral numbers 2 to 19 per cent., where *n* equals 100. This table enables one to ascertain approximately the probable error of all of the coefficients of variation the probable errors of which are not given in the tables.

TABLE 1.

The probable errors (P. E.) for the coefficient of variation (C. V.) from 2 to 19 per cent. where n equals 100.

C. V.	P. E.	C. V.	P. E.	C. V.	P. E.
2.00	.09	8.00	.38	14.00	.68
3.00	.14	9.00	.43	15.00	.73
4.00	.19	10.00	.48	16.00	.78
5.00	.23	11.00	.53	17.00	.83
6.00	.28	12.00	.58	18.00	.88
7.00	.33	13.00	.63	19.00	.93

E. Enumeration of trypanosomes. Several methods have been devised for counting blood-inhabiting protozoa, such as trypanosomes. One of these methods is that of Kolmer (1915), who dilutes the blood with a fluid which hemolyzes the red cells, and then counts the trypanosomes with the ordinary blood counting methods. Ross and Thomson (1911), in their enumerative studies on trypanasomes, made a thick smear of a known quantity of blood, and then, after plasmolyzing the red cells, counted the trypanosomes on the slide. In our work we made red blood cell counts for our various experimental animals, and then made thin, even blood smears from them. After these blood smears were stained, the red blood cells and trypanosomes in a large number of microscopic fields were counted and the ratio between them calculated. Knowing the number of red cells per cmm. and the ratio of trypanosomes to the red cells, it was an easy matter to compute the number of trypanosomes per cmm. of blood. In a number of cases we ity of infections with pathogenic trypanosomes in the rat are of the continuous type. From the standpoint of the resistance built up by the host, these infections present just the opposite conditions to those found in a non-pathogenic trypanosome such as *T. lewisi*. In the following experiments we have studied *T. equiperdum* and *T. rhodesiense*, both of which show this type of infection in the rat.

T. equiperdum: rat 619.

Protocol 619. **Culture rat infected with *T. equiperdum*. Injected intraperitoneally with 1 c.c. of a 1–100 dilution of blood from a guinea pig which had a heavy infection, on February 17, 1921, at 10:00 A.M. Organisms first demonstrated in the blood on February 19, 1921, at 9:00 A.M. Rat died on February 23, 1921, at 8:00 A.M., the last slides being made during terminal convulsions. Trypanosomes were measured and the coefficient of variation calculated for total length, and estimations of the number of organisms per cmm. of blood were made at 8 periods during the course of the infection, as is shown in Fig. 5 and Table 4.**

Incubation period was 1 day 23 hours.

Duration of infection was 5 days 22 hours.

TABLE 4.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 8 intervals during the course of the infection with T. equiperdum in rat 619.

Each statistical constant is based on the measurement of 100 individuals, 800 trypanosomes having been measured during this infection. See Fig. 5 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	2 days 10 hrs.	3 days.	3 days 10 hrs.	3 days 22 hrs.
Mean length	23.640±.117	23.812	22.448	24.552
Standard deviation	1.735±.082	1.788	1.821	1.687
Coefficient of variation	**7.34 ±.35**	**7.51**	**8.14**	**6.87**
Number of trypanosomes per cmm. of blood	**7,000**	**38,000**	**229,000**	**194,000**

Interval after injection.	4 days 12 hrs.	4 days 22 hrs.	5 days 11 hrs.	5 days 22 hrs.
Mean length	24.880	24.792	23.976	23.574
Standard deviation	2.280	2.562	2.191	2.579
Coefficient of variation	**9.16**	**10.33**	**9.13**	**10.94**
Number of trypanosomes per cmm. of blood	**1,333,000**	**1,710,000**	**3,692,000**	**5,102,000**

Even a casual glance at Fig. 5 and Table 4, which give the results obtained for this infection, show that the changes both in the coefficient of variation and the number of parasites are entirely different from those of *T. lewisi.* The coefficient of variation was 7.34 per cent. on the first day after the trypanosomes appeared in the blood. This value is considerably lower than that for the same day in the *T. lewisi* infections. Instead of getting lower, however, as it did in the latter infections, it rose until at the time the animal died it was 10.94 per cent. There is no doubt that this increase in the coefficient of variation is significant from the statistical standpoint, because the difference between the constants in these two days is approximately 6 times its probable error. (10.94 ± .52) — (7.34 ± .35) = (3.60 ± .62). The question then arises is this great difference actually due to an increase in the rate of multiplication of the parasite or simply to some experimental error in technique? This question can not be answered until data for more infections of this kind are obtained. The general shape of the graph, showing the changes in the coefficient of variation, indicates that the rise is significant. We are at least justified in concluding that the host does not build up any resistance which is directed toward a retardation of the reproduction of the parasites, as was the case in the non-pathogenic infection with *T. lewisi.* There is, on the contrary, some evidence that the parasite gradually overcomes more and more the natural resistance which the host had at the beginning of the infection.

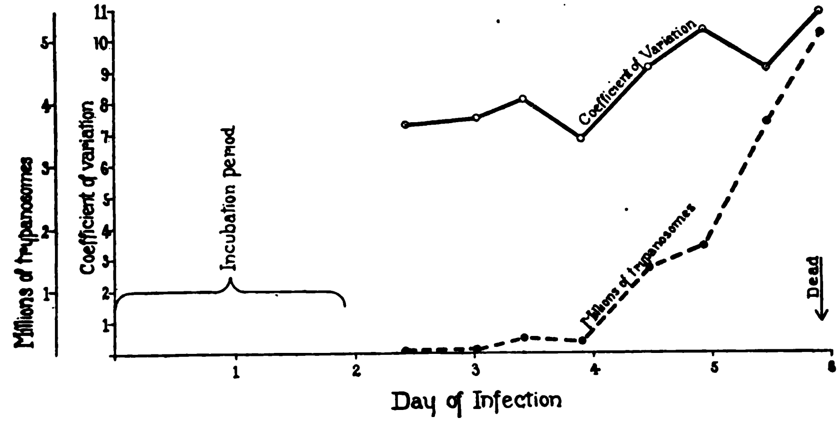

FIG. 5. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. equiperdum* in rat 619.

a production of abnormalities in the trypanosomes due to some adverse environmental factor, such as a trypanolytic antibody. Let us consider the last possibility first. There probably is a trypanolytic antibody formed during the infection, because this is the most plausible way to account for the partial disappearance of the trypanosomes from the blood. This will hardly account for this increase in variability, however, because it is not noticeable in any of the other infections in which a similar antibody is probably formed and, if it were true in this case, we would expect the increase in variability to begin when the organisms first begin to disappear from the blood, namely, on the sixth day. The increase, then, is due to either one of the first two possibilities. From our standpoint, however, this is of little importance, because the host certainly does not build up a resistance which *retards* the rate of reproduction, for the coefficient of variation shows no tendency to decrease in value.

While there is no evidence of a retardation in the reproduction of the parasites, there is evidence of their partial destruction. Between the fourth and sixth days their numbers mounted to 247,000 per cmm. They then rapidly disappeared from the blood, so that there were only 3,000 on the tenth day of the infection. During the next twenty-four hours, they again began to increase, and reached 12,000 shortly prior to the animal's death.

T. rhodesiense: dog 714.

Protocol 714. Very young female puppy, weight 400 grams, infected with *T. rhodesiense.* Injected intraperitoneally with 2 c.c. of a 1–50 dilution of blood from a heavily infected rat, on July 9, 1921. Organisms first demonstrated in the blood on July 11. Dog died on July 18, at 11:00 A.M., the last slides being made one hour before death. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals in each case, and estimations of the number of organisms per cmm. of blood were made 5 times during the course of the infection on the dates shown in Fig. 13 and Table 12.

Incubation period was 2 days.

Duration of the infection was 9 days.

The coefficient of variation for total length (Fig. 13 and Table 12) remained constantly around the value of 9 per cent. throughout the course of this infection. There were indeed no fluctuations which were of statistical significance. While it is unfortunate that no determination of the number of organisms per cmm. was made on the eighth day, it can be seen that the general nature of the changes in the number of parasites was similar to those found in dog 713. Between the fourth and sixth day of the infection, the number of trypanosomes rose until they were 94,000 per cmm. They then dropped to 51,000 on the seventh day and rose again to 61,000 on the ninth day, one hour prior to death.

TABLE 12.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood on 5 days during the course of the infection with T. rhodesiense, in dog 714.

Each statistical constant is based on the measurement of 100 individuals, 500 trypanosomes having been measured during this infection. See Fig. 13 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	4 days.	5 days.	6 days.	7 days.	9 days.
Mean length	26.207±.154	26.492	25.983	24.899	23.612
Standard deviation	2.294±.109	2.388	2.525	2.270	2.227
Coefficient of variation	8.75 ±.42	9.01	9.75	9.11	9.59
Number of trypanosomes per cmm. of blood	7,000	41,000	94,000	51,000	61,000

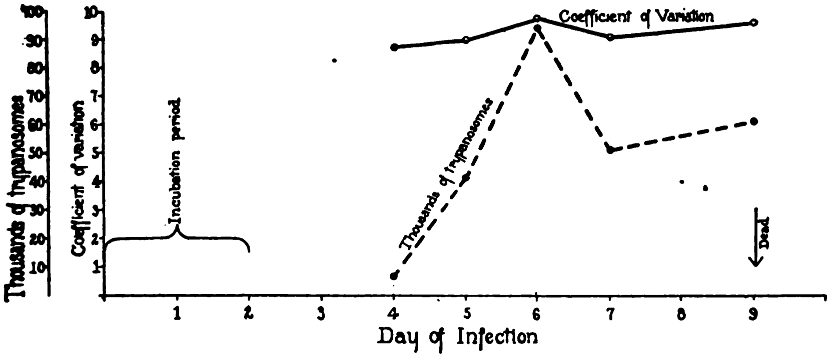

FIG. 13. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. rhodesiense* in dog 714.

To sum up, then, the results from these two dogs indicate that, while there is no resistance that affects the rate of reproduction, there is a marked resistance developed which is directed toward a destruction of the parasites after they are formed. It is significant to note that the development of this resistance apparently had little or no connection with the time of death. While the death of both animals took place when the trypanosomes were actually increasing in number, the number of parasites was low in comparison to the number present at the peak of the infection. This is in marked contrast to the condition of affairs in rats and mice (see especially the discussion under rat 709).

THE GUINEA PIG AS A HOST OF TRYPANOSOME INFECTION.

One of the best examples of relapsing types of infection is found when the pathogenic African trypanosomes are grown in the guinea pig. They are generally of a more chronic type than the infection in any of the previous hosts considered. A description of their general character can be found in Pearce and Brown (1918). The following experiments give an account of two of these infections.

A. Pathogenic infections of a relapsing type.

T. rhodesiense: guinea pig 719.

Protocol 719. Guinea pig, infected with *T. rhodesiense.* Injected intraperitoneally with 2 c.c. of a 1–200 dilution of blood from a heavily infected rat, on July 13, 1921. Organisms first demonstrated in the blood on July 18. Guinea pig was dead on August 25, at 9:00 A.M. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals in each case, and estimations of the number of organisms per cmm. of blood were made 19 times during the course of the infection, as is shown in Fig. 14 and Table 13. From the 5th to the 23d day of the infection only one or two trypanosomes could be found in fresh blood preparations. The infection during this period was so light that the organisms could be neither counted nor drawn.

Incubation period was 5 days.

Duration of the infection was approximately 42½ days.

This infection was a fairly long one, lasting for forty-three days (Fig. 14 and Table 13). After an incubation period of five days, a single trypanosome was demonstrated in the blood. From the fifth to the twenty-third day, one or two organisms could be found in fresh blood preparations, but they were never in great enough numbers, either to obtain a sample for the computation of the coefficient of variation, or to get an estimation of their numbers. This period of the infection undoubtedly represents a period of resistance by the host, but we have no way of telling whether the failure of the parasites to increase in number was due to a period of low reproductive activity or to the destruction of a large part of their numbers by the host.

not be accounted for on the basis of the cessation of reproductive activity alone, because, had this been the only factor involved, the number of parasites would have remained stationary. We must, therefore, conclude that, at this point, the host has built up a resistance not only toward a retardation of the rate of reproduction, but also toward an actual destruction of the parasites after they are formed. From the fourteenth day until the thirty-fifth day, the number of parasites in the blood remained practically constant with the exception of a slight decrease. Between the thirty-fifth and thirty-sixth days they completely disappeared from the blood and could never be found again although repeated attempts were made to demonstrate their presence.

To sum up the results obtained in this infection with *T. lewisi* in the rat, we find: (1) A resistance was built up which retarded the rate of reproduction of the parasites more and more, and finally inhibited it altogether (by the tenth day). It is true that growth of the organisms may have taken place for some days more, but apparently all actual multiplication was stopped by this time. (2) Between the tenth and fourteenth days, a resistance was developed which killed off a large number of the parasites, i.e., it was directed toward their destruction after they were formed. Finally, (3) a resistance of the second type was again developed by the thirty-fifth day which caused the complete destruction of all the remaining organisms.

In the present paper we will limit our discussion simply to the different *effects* of the host's resistance and not its *nature*. It is possible that these different effects are due to the same fundamental type of resistance. This is notably true of the first and second effects given above. We will call attention to the fact, however, that the agency which finally destroys the trypanosomes at the end of the infection and that which causes the first decline in numbers is probably different. We feel this to be true, because the time which elapses between the two varies to such an extent. In the present infection this interval was about twenty-five days, but it may vary from a week to several months.

Rat 116.

Protocol 116. Culture rat infected with *T. lewisi*. Injected intraperitoneally with a single trypanosome, which was isolated with a Barber pipette, on April 8, 1920. Organisms first demonstrated in the blood on April 16, 1920. Trypanosomes disappeared from the blood on June 26, 1920, and were never found after this date, although repeated blood examinations were made. No clinical symptoms were observed during the course of the infection. Trypanosomes were measured and the coefficient of variation for total length calculated on 6 days, as is shown rat, on May 11, 1921, at 2:00 P.M. Organisms first demonstrated in the blood on May 13, at 2:00 P.M. Rat was dead by May 25. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals, and estimations of the number of organisms per cmm. of blood were made 9 times during the course of the infection, on the dates shown in Fig. 8 and Table 7.

Incubation period was 2 days.

Duration of the infection was approximately 13½ days.

TABLE 7.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 9 intervals during the infection with T. rhodesiense in rat 709.

Each statistical constant is based on the measurement of 100 individuals, 900 trypanosomes having been measured during this infection. See Fig. 8 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	2 days 22 hrs.	4 days 20 hrs.	5 days 20 hrs.	7 days 1 hr.	7 days 22 [illegible] hrs.
Mean length	26.509±.163	26.956	25.485	24.489	24.744[illegible]4
Standard deviation	2.430±.115	3.301	2.934	2.456	2.569[illegible]9
Coefficient of variation	**9.16 ±.44**	**12.24**	**11.51**	**10.02**	**10.38**
Number of trypanosomes per cmm. of blood	**88,000**	**750,000**	**698,000**	**850,000**	**631,00[illegible]0**

Interval after injection.	8 days 21 hrs.	9 days 21 hrs.	11 days 20 hrs.	13 days 2 hrs.
Mean length	23.894	24.603	25.445	24.575
Standard deviation	2.259	2.432	2.846	2.665
Coefficient of variation	**9.45**	**9.88**	**11.18**	**10.84**
Number of trypanosomes per cmm. of blood	**956,000**	**613,000**	**1,867,000**	**2,388,000**

This infection is interesting because it lasted twice as long as the previous infections and may, therefore, give us some insight into why some rats live longer with an infection of these pathogenic trypanosomes than others. The coefficient of variation for total length was 9.16 per cent. on the first day that the parasites appeared in the blood, and it stayed somewhere in the neighborhood of 10 per cent. for the rest of the infection. We do find that the value on the first determination (9.16 ± .44) is significantly lower than that at the second determination (12.24 ± .59). This difference is 3.08 ± .73 and is about four times its probable error. This second determination is, howvious experiment in a striking manner. The coefficient of variation for total length on the second day after the trypanosomes appeared in the blood (the ninth day after infection) was very high (26.80 per cent.), but it rapidly decreased, so that it was only 3.21 per cent. on the thirty-second day, from which value it probably did not change throughout the rest of the infection. It is true that there was a slight increase on the seventy-ninth day, but this change is so small in comparison with the changes noted at the beginning of the infection that it can probably be looked upon as an experimental error, rather than any actual change in the trypanosomes. These results indicate that the host has built up a resistance which is directed toward a retardation of the rate of reproduction of the parasites, so that by the thirty-second day of the infection all reproduction has ceased. This is similar to the results obtained for rat 105 except that a longer time elapsed before all reproduction was inhibited—a result probably connected with the fact that this infection was started with a single organism.

The changes in the number of trypanosomes per cmm. are also closely similar to those observed in rat 105. There was an initial rapid rise in the number of trypanosomes so that there were 504,000 on the twenty-fifth day of the infection. From the twenty-fifth day to the thirty-ninth day, they disappeared from the blood at a rate almost equal to their initial rise, so that by the latter day there were only 68,000 per cmm. From the thirty-ninth day to the seventy-ninth day the decline was very much more gradual. On the seventy-ninth day, however, there were only 12,000 per cmm. in the blood, and within the next twenty-four hours these totally disappeared, never to reappear. Just as in rat 105, then, there is a resistance built up which is directed toward a destruction of the parasites causing a marked decrease at the end of the multiplicative period and their total disappearance at the end of the infection:

Our general results in regard to the changes in the number of trypanosomes per cmm. of blood during the course of infections with *T. lewisi* are similar to those obtained by Steffan (1921).* This author, however, did not consider these changes from the standpoint of the present work.

B. Pathogenic infections of a continuous type. By far the major-length of the infection. Here again there is little evidence that there was any marked progressive change in the coefficient of variation for total length. The true value of this constant was about 10 per cent throughout the course of the infection (Fig. 15 and Table 14). Between the fifth and sixth days of the infection the parasites increased until they numbered 2,000 per cmm. of blood. They then disappeared from the peripheral circulation and were not found between the seventh and twelfth days. They reappeared on the thirteenth day and increased until their number was 17,000 per cmm. Here again, they diminished to 800 on the fifteenth day and rose to 5,000 on the sixteenth day. This was followed by a complete disappearance from the blood between the eighteenth and twentieth days. Their next appearance in the blood, however, was their last, and their numbers rapidly rose to 252,000 on the twenty-sixth day, just before the death of the guinea pig. In this infection, therefore, we have four periods of increase in the number of the parasites and three periods of decrease, two of which resulted in their complete disappearance from the peripheral circulation.

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* Steffan also describes an acute type of infection with T. lewisi in which the parasites increase in the blood until the host dies. Recently we have found similar lethal infections with this trypanosome and are studying them with the methods given in the present paper.

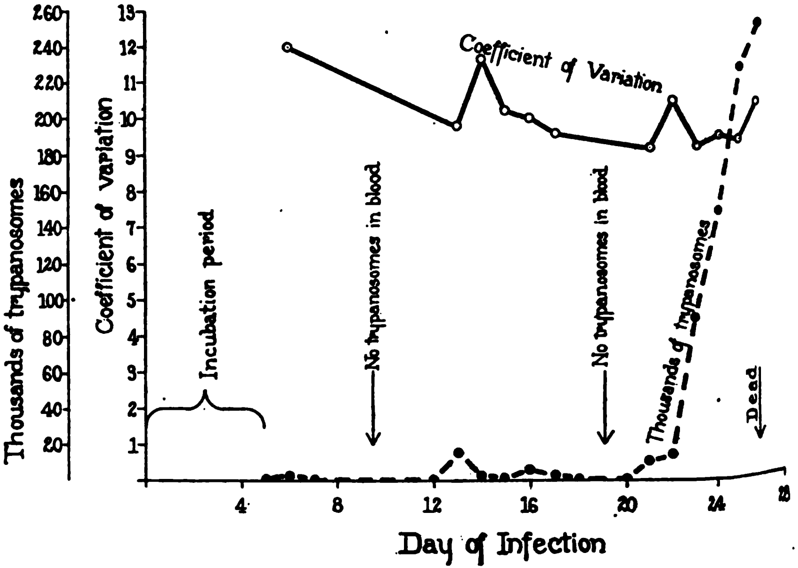

FIG. 15. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection of *T. brucei* in guinea pig 721.

Even a casual glance at Fig. 5 and Table 4, which give the results obtained for this infection, show that the changes both in the coefficient of variation and the number of parasites are entirely different from those of *T. lewisi.* The coefficient of variation was 7.34 per cent. on the first day after the trypanosomes appeared in the blood. This value is considerably lower than that for the same day in the *T. lewisi* infections. Instead of getting lower, however, as it did in the latter infections, it rose until at the time the animal died it was 10.94 per cent. There is no doubt that this increase in the coefficient of variation is significant from the statistical standpoint, because the difference between the constants in these two days is approximately 6 times its probable error. (10.94 ± .52) — (7.34 ± .35) = (3.60 ± .62). The question then arises is this great difference actually due to an increase in the rate of multiplication of the parasite or simply to some experimental error in technique? This question can not be answered until data for more infections of this kind are obtained. The general shape of the graph, showing the changes in the coefficient of variation, indicates that the rise is significant. We are at least justified in concluding that the host does not build up any resistance which is directed toward a retardation of the reproduction of the parasites, as was the case in the non-pathogenic infection with *T. lewisi.* There is, on the contrary, some evidence that the parasite gradually overcomes more and more the natural resistance which the host had at the beginning of the infection.

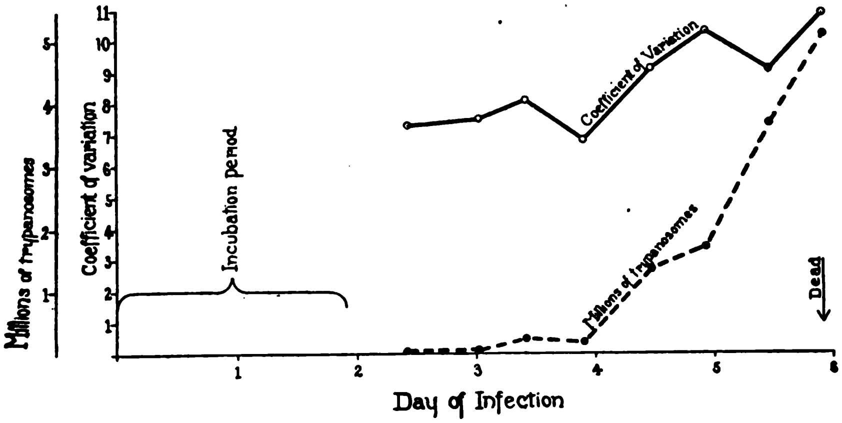

FIG. 5. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. equiperdum* in rat 619.

for almost any animal. Take, for example, man. If we make a comparison of the variability of some measure of size, such as height, between a population in which no reproduction is taking place and one in which rapid reproduction is occurring, the former will consist largely of adults who will be more or less uniform in height, while the latter will consist of adults and children in all stages of growth and will exhibit a very much greater variability in height. It is also apparent that the greater the number of children (the higher the rate of reproduction) the greater will be the variability. In trypanosomes where each division results in the formation of one long and one short individual, the same reasoning will hold in regard to the variability in total length.

B. Observational evidence for trypanosomes. It must not be supposed, however, that our results are based entirely on this *a priori* reasoning, sound as it may seem. In the experiments on *T. lewisi* in the rat one can satisfy oneself of the general validity of the conclusions by an examination of the slides. Take, for example, rat 105. The slides on the fourth day of the infection contain many organisms in the process of division. These evidences of division decreased until one could never find any but typical " adult " trypanosomes, after the twenty-eighth day. In fact, our general results in regard to the rate of reproduction in *T. lewisi* have been known for some time, as is shown by the work of Minchin (1912) and Minchin and Thomson (1915). Previous investigators determined the length of the multiplicative stage of the infection by just such an examination of the slides as we have outlined above. In this connection Minchin and Thomson (1915) say: " So long as the trypanosomes are multiplying in the rat's blood, they are of various sizes, some of the ordinary, normal size, others very small, and others again much above the normal size. Marked variation in the size of the trypanosomes is a sure sign that multiplication is proceeding, even when actual division-stages are so scarce in the preparation that prolonged search is necessary in order to find them. As soon as the multiplication is ended the trypanosomes are all of one type and size, allowing for slight individual variations that are not perceptible without careful measurement; to such trypanosomes, the normal form of *T. lewisi* and the sole form occurring in the blood when once the multiplication is at end, we shall refer always as ' ordinary.' " We give this quotation from Minchin and Thomson not only to show that they knew the general facts which we have outlined in regard to the changes in the rate of reproduction

TABLE 5.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 4 intervals during the infection with T. rhodesiense in rat 703.

Each statistical constant is based on the measurement of 100 individuals, 400 trypanosomes having been measured during this infection. See Fig. 6 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	1 day 20 hrs.	2 days 20 hrs.	3 days 20 hrs.	5 days 20 hrs.
Mean length	25.733±.159	26.418	27.328	26.196
Standard deviation	2.368±.112	2.479	3.284	3.238
Coefficient of variation	9.20 ±.44	9.38	12.01	12.36
Number of trypanosomes per cmm. of blood	31,000	198,000	702,000	3,158,000

indicating that the parasites multiplied more rapidly at the end of the infection than at the beginning. We are, therefore, justified in concluding that not only does the host not build up any resistance which

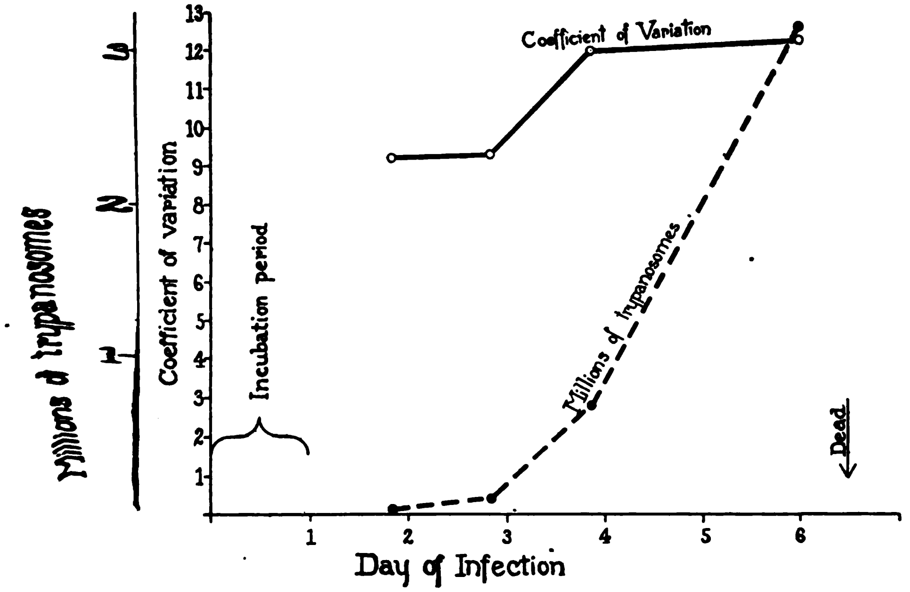

FIG. 6. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. rhodesiense* in rat 703.

these measurements into five groups which correspond to the stages in, what we might call, the life-history of the individual trypanosome. The individuals are placed in the separate groups, according to their stage in division as follows:* (1) all forms possessing a single parabasal body and nucleus, (2) forms possessing two parabasal bodies, but a single nucleus, (3) forms having two parabasal bodies and the nucleus in the process of division, (4) forms possessing two parabasal bodies and two nuclei, but showing no evidence of division of the body, and (5) forms with the body in actual process of division. It is to be emphasized that the first class, although it contains forms which are not dividing, is a very much more heterogeneous class than the others, and should show a greater range of variation. This follows from the fact that it contains not only "adult" forms, but the

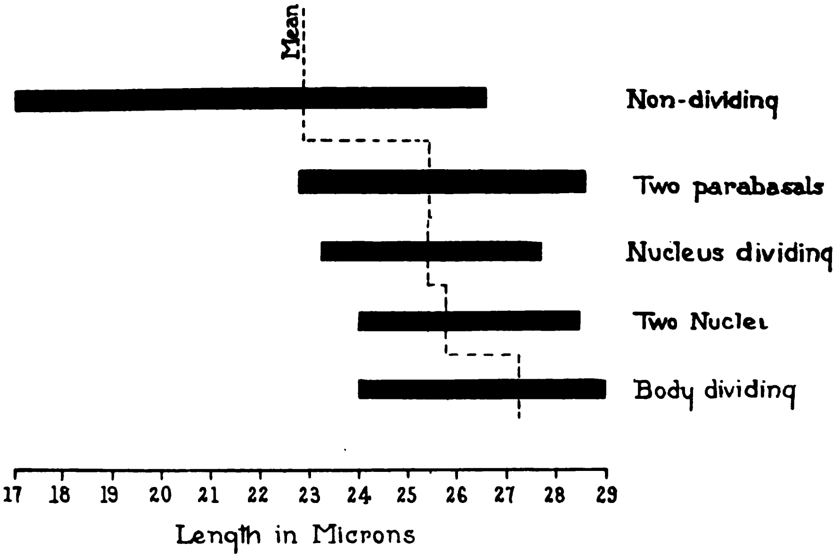

FIG. 16. Bar diagram showing the range of variation in total length between sets of *T. equiperdum* which (1) show no signs of division, (2) possess two parabasal bodies and one undivided nucleus, (3) possess two parabasal bodies and one nucleus in the process of division, (4) possess two parabasal bodies and two nuclei, and (5) show the body in actual process of division. The broken line indicates the mean of each group. For full explanation see text.

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* The 260 individuals do not represent a random sample of the general population, but each class is itself a random sample of the trypanosomes in that stage of division. In other words, the number of individuals in each class does not represent the true proportion of the individuals in that stage of division, as found in a random sample of the general population.

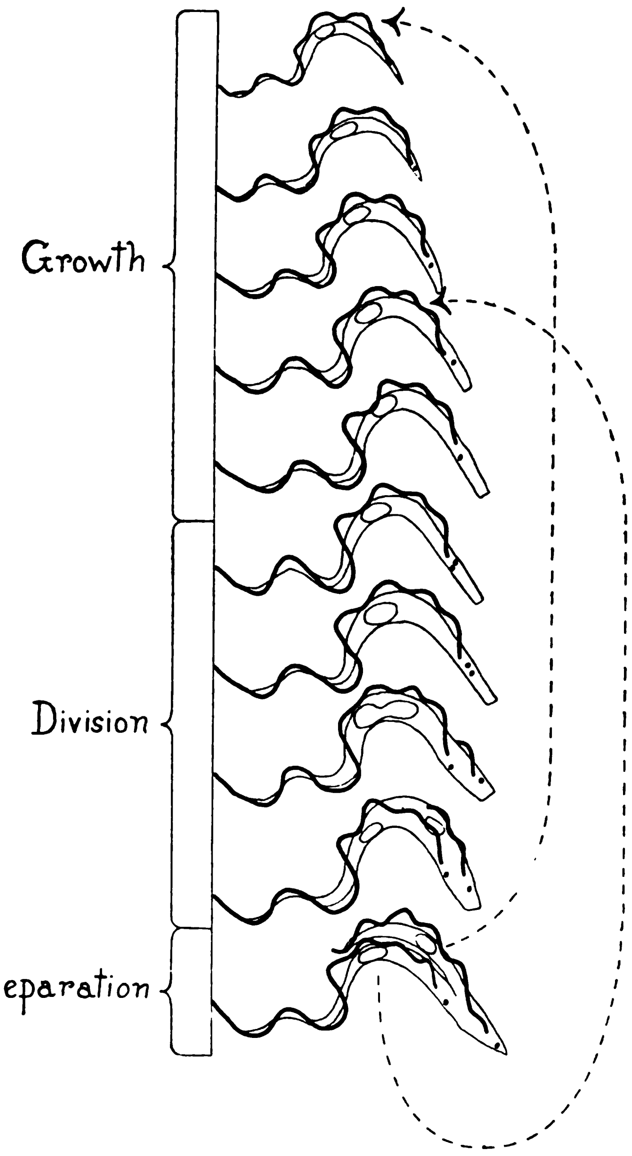

Fig. 17. Diagram representing the probable course of events in asexual production in *T. equiperdum*. This diagram is based on the results given in e text and in Fig. 16.

rat, on May 11, 1921, at 2:00 P.M. Organisms first demonstrated in the blood on May 13, at 2:00 P.M. Rat was dead by May 25. Calculations of the coefficient of variation for total length, based on the measurement of 100 individuals, and estimations of the number of organisms per cmm. of blood were made 9 times during the course of the infection, on the dates shown in Fig. 8 and Table 7.

Incubation period was 2 days.

Duration of the infection was approximately 13½ days.

TABLE 7.

Mean, standard deviation, and coefficient of variation for total length, and the number of trypanosomes per cmm. of blood at 9 intervals during the infection with T. rhodesiense in rat 709.

Each statistical constant is based on the measurement of 100 individuals, 900 trypanosomes having been measured during this infection. See Fig. 8 for graph of the coefficient of variation and the number of trypanosomes per cmm. of blood.

Interval after injection.	2 days 22 hrs.	4 days 20 hrs.	5 days 20 hrs.	7 days 1 hr.	7 days 22 hrs.
Mean length	26.509±.163	26.956	25.485	24.489	24.744
Standard deviation	2.430±.115	3.301	2.934	2.456	2.569
Coefficient of variation	**9.16 ±.44**	**12.24**	**11.51**	**10.02**	**10.38**
Number of trypanosomes per cmm. of blood	**88,000**	**750,000**	**698,000**	**850,000**	**631,000**

Interval after injection.	8 days 21 hrs.	9 days 21 hrs.	11 days 20 hrs.	13 days 2 hrs.
Mean length	23.894	24.603	25.445	24.575
Standard deviation	2.259	2.432	2.846	2.665
Coefficient of variation	**9.45**	**9.88**	**11.18**	**10.84**
Number of trypanosomes per cmm. of blood	**956,000**	**613,000**	**1,867,000**	**2,388,000**

This infection is interesting because it lasted twice as long as the previous infections and may, therefore, give us some insight into why some rats live longer with an infection of these pathogenic trypanosomes than others. The coefficient of variation for total length was 9.16 per cent. on the first day that the parasites appeared in the blood, and it stayed somewhere in the neighborhood of 10 per cent. for the rest of the infection. We do find that the value on the first determination (9.16 ± .44) is significantly lower than that at the second determination (12.24 ± .59). This difference is 3.08 ± .73 and is about four times its probable error. This second determination is, however, the only one which differs significantly from the remaining constants obtained, and we seem justified, therefore, in concluding that the coefficient of variation for total length, and, in consequence, the rate of multiplication of the trypanosomes remains constant throughout the infection.

Turning now to a consideration of the changes in the numbers per cmm., we find a different picture than in the infections of shorter duration. During the first days of the infection the number of parasites rose rapidly until it reached 750,000 per cmm. by the fifth day. Instead of increasing continuously, as in the previous infection, they then remained practically constant in number until the tenth day, when there were 613,000 per cmm. On the tenth day they began to increase again and numbered 2,388,000 per cmm. shortly before the death of the host. We have, therefore, clear evidence of a resistance being developed between the fifth and tenth days of the infection, which was directed solely toward the destruction of the parasites, and was apparently without effect upon the rate of reproduction of

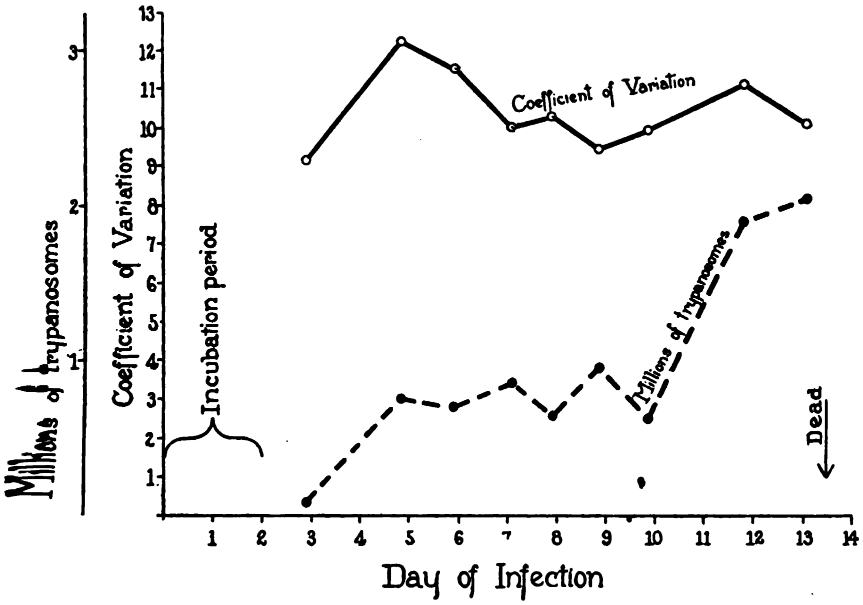

FIG. 8. Graph showing the coefficient of variation for total length (comparative measure of the rate of reproduction) and the number of trypanosomes per cmm. of blood, throughout the course of the infection with *T. rhodesiense* in rat 709.

other factors beside growth and reproduction might not influence the coefficient of variation for total length. Investigators working with free-living species of protozoa have noted that such things as temperature, food supply, the addition of chemicals to the culture medium, etc. (see especially Erdmann, 1920) increase the variability in size of populations. Most of these environmental factors are known to affect the rate of reproduction, and it seems very probable that their effect on the variability in size may be due to their effect on the rate of reproduction. In any event most of these factors are usually constant from day to day for a parasite living in mammalian blood.

There are, however, other environmental factors which might increase variability and size without increasing the rate of reproduction. Suppose, for example, that a trypanolytic antibody is formed within the host. In killing the organisms this lysin might produce many distortions in shape and size entirely independent of growth. In the numerous cases presented in the preceding experiments where the trypanosomes suddenly decreased in number or disappeared from the blood, such a trypanolytic antibody is probably formed and yet in none of these cases is there any evidence of an increase in the coefficient of variation for total length. The sudden rise of the coefficient of variation on the tenth day in dog 713 might be considered an exception to this statement. We do not think, however, that this increase in variability is due to the formation of a trypanolytic antibody because: first, it is the only case of such an increase among six experiments in which there is an effect on the number of trypanosomes and in which, presumably, a trypanolytic antibody is formed; and second, if this increase had been due to a trypanolytic antibody, it would have begun when the effect of the lytic agent first became apparent, i.e., on the seventh day.

The production of a trypanolytic antibody might conceivably affect the variability in size in just the opposite direction, in that it might lower the coefficient of variation due to the selective killing of trypanosomes of a specific size. It might, for example, kill only the young forms and leave the "adults." If such selective killing occurred, we would not only expect a decrease in the coefficient of variation, but also a marked change in the mean size. It is evident that the latter is true because the mean would be lowered or raised according to whether the trypanolytic antibody selected the large "adult" forms or the small young forms. An examination of the data for the pathogenic forms reveals the fact that not onlv is the coefficient of variation for total length constant, but the mean length shows very few changes throughout the course of each infection.

While there is no evidence of any selective action on the part of the destructive agent for the pathogenic trypanosomes, this is not as apparent in the two infections with *T. lewisi.* In these infections we not only find changes in the coefficient of variation throughout the infection, but we also find changes in the mean length. Let us first consider the coefficient of variation. During the first part of the infection, this rapidly decreases in value. This decrease, however, cannot be due to the selective killing of the trypanosomes, because by far the greater part of the decrease takes place *before* the trypanosomes begin to disappear from the blood. The mean length (see especially rat 105) increases during the first part of the infection, but in this case the entire increase takes place before the trypanosomes begin to disappear. In both cases, therefore, the changes seem to be independent of the disappearance of the trypanosomes from the blood, and, therefore, independent of the action of the trypanolytic antibody. Finally, in this connection, it is well to repeat that our conclusions in regard to the rate of reproduction of *T. lewisi* in the rat, which were drawn from a study of the changes in the coefficient of variation for total length, have been checked and verified by numerous direct observations.

Discussion of Results.

A brief discussion of some of the fundamental factors involved in the resistance of a host to trypanosome infections, as well as the results obtained in our work, will enable us to understand more clearly the conclusions drawn in this paper. We have used the term resistance to denote collectively those factors, either active or passive, which operate adversely against the invading organism. Our particular interest in the resistance of the host to trypanosomes is in that resistance which develops after the organisms are established in the host. We have not taken into consideration any of the great questions of the resistance of various hosts to invasion. Considering the former type of resistance from the logical standpoint, it seems to us evident that this may be divided into those factors which tend to retard the rate of reproduction of the parasite and those which tend to actually destroy them after they are formed. Both of these factors will, of course, affect the number of organisms present in the blood at any given time. Taking these facts into consideration we have been able to differentiate between them by making frequent determinations of the number of organisms per cmm. of blood and of the coefficient of variation for total length, which gives us a comparative measure of the rate of reproduction of the trypanosomes, regardless of the number destroyed in consequence of resistance. The rationale of this general procedure is given on page 267 and a discussion of the validity of the coefficient of variation for total length as a comparative measure of the rate of reproduction is given on page 305.

Before leaving the question of the coefficient of variation for total length, it is well to call attention to the fact that in our tables we have given not only the actual coefficient, but the mean length and standard deviation from which the coefficient of variation was calculated. Incidentally, these tables contain some very valuable data in regard to the effect on total length, of growing the same strain of trypanosomes in different hosts. We will not consider this question in the present study, but will reserve it for a later paper.

Turning now to a consideration of the resistance which a host offers toward a trypanosome infection after the parasites have established themselves, we find that three different conditions have been brought out by the preceding experiments. In the first place, the highest grade of resistance was found in the non-pathogenic *T. lewisi* in the rat. Here the host builds up an ever increasing resistance which retards the rate of reproduction of the trypanosomes. This resistance is so pronounced that, during the last (or "adult") stage of the infection, the parasites exist in the blood in the so-called "adult" condition, without exhibiting any signs of reproduction or growth. We also find in this case that just as the resistance against the rate of reproduction is reaching its climax (viz., 10th day in rat 105), the host suddenly develops a resistance which destroys a majority of the organisms in the blood. The remaining parasites live in the blood for varying lengths of time (sometimes several months) until they are suddenly destroyed as the result of the final resistance which the host builds up against them. After their disappearance from the blood, the host is immune to another infection of *T. lewisi* (see Laveran and Mesnil, 1912). In the second place, we find just the opposite condition of affairs in most infections of rats and mice due to pathogenic trypanosomes. Here (with the exception of long infections, such as rat 709 and rat 729) we can find no evidence that any resistance is built up either toward the rate of reproduction or toward the destruction of the parasites, after they are formed. Once the trypanosomes establish themselves in the host, they reproduce and live in the blood unmolested. In some cases, indeed, there is evidence that they reproduce more rapidly as the infection progresses (rats 619 and 703). In the third place, we find in the relapsing type of infection (pathogenic trypanosomes in the dog and guinea pig and *T. equinum* in the rat) that no resistance is built up which retards the rate of reproduction of the trypanosomes, but a resistance does arise which destroys a large number of the parasites after they are formed.

The fact that resistance in the non-pathogenic *T. lewisi* produces two different effects whereas resistance in pathogenic species (if present at all) produces only one of these effects indicates that in the non-pathogenic forms there is either an entirely different type of mechanism of resistance, or that there is a different type of mechanism superimposed on the mechanism found in the pathogenic forms. It is interesting to note that Miss Robertson (1912) has brought forward considerable evidence to show that in *T. gambiense* in the monkey there is an endogenous cycle, in which phases of active reproduction alternate with phases of little or no reproduction. If this be true, it would indicate that this form in the monkey encounters much the same type of resistance as *T. lewisi* in the rat, except that in the latter case, when reproduction once ceases it never starts again. Miss Robertson's results indicate that *T. gambiense* in the monkey encounters a resistance midway between the type we have described in *T. lewisi* in the rat and the pathogenic species in the guinea pig. Such a result would not be at all surprising, and with the growing evidence that *T. gambiense* can exist in certain mammals as a non-pathogenic parasite it may be possible to find a host in which this trypanosome encounters the same type of resistance as *T. lewisi* in the rat.

In considering the resistance encountered by the pathogenic trypanosomes, the question immediately arises what effect the development of a resistance has on the time of death of the host. In the case of these trypanosomes in the rat we found one case (*T. rhodesiense* in rat 709) which strongly suggested that the development of a resistance tending to destroy the parasites actually prolonged the life of the host. In other words, there seems to be a definite connection in rats, and probably mice, between the number of organisms in the blood and the time of death of the host. This correlation does not, however, seem to hold good when the same trypanosomes are grown in the dog and guinea pig. In our two experiments on dogs, both animals died when the number of trypanosomes in the blood was very much less than it was at the peak of the infection. Of the two guinea pigs one died at a time when the number of trypanosomes in the blood was at its maximum height, whereas the other died when they were comparatively few in number. Of course, we can not say that the development of a resistance in the latter hosts did not lengthen the life of the animal. The results themselves, however, do not reveal any obvious connection between the two factors as was suggested in the case of *T. rhodesiense* in rat 709.

In our preceding discussion we have assumed that a disappearance of the trypanosomes from the peripheral blood indicates that they have been actually destroyed by the host and not that they have migrated into the tissues. As has already been pointed out, this seems to be a safe assumption to make, because Levaditi and McIntosh (1910) have demonstrated the presence of a trypanolytic antibody after the crises in such infections. Then, too, if the disappearance from the blood were to be explained by a migration into the tissues, there should be a great many more parasites in the tissues during the period of low blood infection. At the present time, there is not the slightest evidence that this is true. The same conclusion was reached by Miss Robertson (1912), although on slightly different premises. Of course, had we worked with *T. cruzi,* the condition of affairs would have been different, because this trypanosome shows an entirely different behavior and method of reproduction than do any of the other trypanosomes. In fact, the most typical form of this parasite, in the vertebrate host, is an almost true tissue-inhabiting Leishmania.

We feel that, in the present work, we have been able to introduce a new method of attack on some of the problems of resistance. This method, however, deals with the *effect* of the resistance, and not with its *mechanism.* What might constitute the most probable explanation of the effects which we have observed would necessitate a lengthy discussion. This, however, had best wait until we can do some actual experimental work on the question. We will point out, however, that the two different types of resistance in *T. lewisi* may be connected with the same fundamental mechanism.

In the future, we hope to continue the study of resistance and extend our method, if possible, to other infections. It is obvious that it can not be used unless accurate measurements of the size of the parasites can be made. This can be done for a number of protozoa, but it is impracticable for most of the bacteria. It is possible, however, that the spirochetes will lend themselves to this method of attack.

GENERAL SUMMARY.

1. The conclusions reached in this paper are based on the study of 13 experimental infections with trypanosomes, divided as follows: *T. lewisi* in the rat (2), *T. equiperdum* in the rat (1), *T. equinum* in the rat (1), *T. brucei* in the dog (1), mouse (1), and guinea pig (1), and *T. rhodesiense* in the rat (3), mouse (1), dog (1), and guinea pig (1). In the study of these infections determinations of the number of trypanosomes per cmm. of blood were made on 125 days and drawings, measurements, and computations of statistical constants were made for 114 sets of 100 trypanosomes each.

2. The resistance which a host builds up toward a trypanosome infection may do one or both of two things: (*a*) it may retard the rate of reproduction of the parasites, or (*b*) it may destroy the parasites after they are formed.

3. These two conditions can be differentiated by ascertaining: (*a*) the changes in the number of parasites per cmm. of blood, supplemented by (*b*) the changes in the coefficient of variation for total length of the trypanosomes, which gives a comparative measure of their rate of reproduction, irrespective of the number destroyed in consequence of the resistance. These two determinations differentiate between the two types of resistance because:

The number of trypanosomes per cmm. of blood = the number produced by reproduction of the parasites — the number destroyed in consequence of the resistance.

4. The validity of the coefficient of variation for total length, as a measure of the rate of reproduction of trypanosomes, is discussed in detail on page 305. Its great value lies in the fact that it measures the rate of reproduction irrespective of the number destroyed by the resistance.

5. Three conditions have been found in regard to the resistance to trypanosome infections.

(*a*) In the non-pathogenic *T. lewisi* in the rat, there is a resistance built up which is directed both toward a retardation of the rate of reproduction of the parasites, and toward their actual destruction after they are formed (rats 105 and 116).

(*b*) In the continuous infections: *T. rhodesiense* (with the exception of the long infection in rat 709), *T. brucei, T. equiperdum* in the rat, and *T. brucei* and *T. rhodesiense* in the mouse, there is no evidence of any resistance being built up, either toward the rate of reproduction or toward the destruction of the parasites, after they are formed. In two of these infections (rat 619 and rat 703), there is some evidence that the rate of reproduction of the parasites increases as the infection progresses (rats 703, 705, 709, and 619, and mice 728 and 724).

(*c*) In the relapsing infections: *T. equinum* in the rat, *T. rhodesiense* and *T. brucei* in the dog and guinea pig, the host builds up a resistance directed toward a destruction of the parasites, after they are formed, but it does not affect the rate of reproduction of the remaining parasites (rat 729, dogs 713 and 714, and guinea pigs 719 and 721).

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GIARDIA ENTERICA: A PARASITIC INTESTINAL FLAGELLATE OF MAN.

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GIARDIA ENTERICA: A PARASITIC INTESTINAL FLAGELLATE OF MAN.*

By CHARLES E. SIMON, B.A., M.D.

Plates XXII to XXIV.

(Received for publication, May 18th, 1921.)

Introduction.

In one of his early papers on human giardiasis Grassi (1881) expressed the opinion that the infestation of man was in all probability brought about by the ingestion of food contaminated with the feces of correspondingly infested rodents. He was led to this conclusion by the discovery of organisms in the common house mouse (*Mus musculus*), the black rat (*Mus rattus*), the Norwegian rat (*Mus decumanus*), the meadow mouse (*Arvicola arvalis*) and the wood mouse (*Arvicola silvestris*), which he evidently regarded as identical with those occurring in the human being. He also found giardias in cats, dogs, sheep and rabbits, but apparently did not regard these as important agents in the production of the human infestation. He mentions particularly that cats are rarely infested, but makes no statement regarding the frequency of the occurrence of the parasite in dogs, sheep and rabbits. His belief that the rodents in question are responsible for the human infestation is based upon the frequency of the infestation of these animals and the common custom among the peasants of northern Italy of keeping their bread in open granaries, where rats and mice have ready access to it and can contaminate it with their feces. He mentions that he has frequently noted this himself.

The doctrine that the human infestation is probably of rodent origin has been handed down in the text-books, ever since, notwithstanding the fact that no proof of the identity of the forms found in man and those occurring in animals, nor indeed of the animal forms amongst each other, has ever been adduced. The entire problem of giardiasis in the human being has, indeed, received but scant attention

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* From the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

The present paper is the first of a series which the writer is about to present on the general subject of Giardiasis.

While the coefficient of variation for total length is as valuable an indicator of the rate of reproduction in the pathogenic forms as in *T. lewisi,* it is interesting to note that the actual changes in total length resulting from division are somewhat different in the two cases. The foregoing evidence indicates that in the pathogenic trypanosome, *T. equiperdum,* once an organism has grown to its "adult" size, it does not increase appreciably in length during the actual process of division. The higher variability resulting from division is due chiefly o growth of young forms. In *T. lewisi* we not only have this factor, ut the "adult" trypanosomes apparently increase in length just rior to division. That this is true is indicated by a comparison of amples *A* and *B* in Fig. 1. Here it can be seen that one of the inividuals in the rapidly reproducing population is considerably longer han any of the "adult" forms from the non-reproducing population.

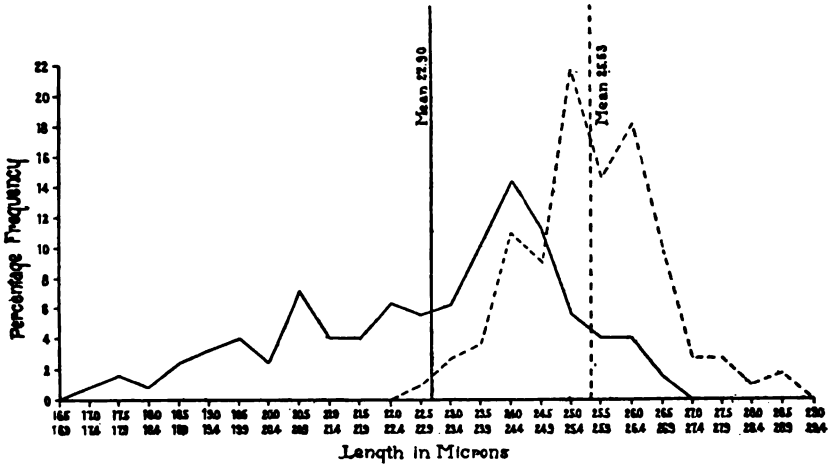

FIG. 18. Frequency polygon showing the variation of those forms which show no signs of division, and which include forms of all ages (solid line), as compared with those actually in the process of division which are "adults" (broken line).

*C. Other factors besides growth and reproduction which might ffect the variability in size.** The question might be raised whether rodents, under the name *Dimorphus muris*. He expressed the opinion that this organism was identical with one which he had encountered a short while before in the human being. Two years later (1881) he abandoned the term *Dimorphus muris* and named the parasite *Megastoma entericum.* Bütschli (1886) in reviewing the taxonomic status of the organism subsequently changed this to *Megastoma intestinalis,* while Blanchard (1888) suggested the name *Lamblia intestinalis* to commemorate the name of its actual discoverer. Stiles (1902) still later changed Blanchard's species name to *duodenalis*. Kofoid (1915) finally proposed the name *Giardia lamblia* Stiles in *litt.* for the same form, and still more recently (1920) changed this to *Giardia enterica,* which, according to the rules of nomenclature, would be correct, providing that the mammalian genus Giardia is, indeed, identical with that originally described in tadpoles, and to which Künstler in 1882 and 1883 first applied the name *Giardia.*[*]

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* We have shown elsewhere (Taliaferro, 1921 *b*) that passage of *T. lewisi* hrough the invertebrate host (in contradistinction to direct passage through he vertebrate host) increases variability, and that this increase in variability is not due to any phenomenon of growth. This, however, has no bearing on the present paper where all work was done with trypanosomes in the vertebrate host.

HISTORICAL SURVEY OF THE EVOLUTION OF OUR KNOWLEDGE OF THE MORPHOLOGY OF GIARDIA ENTERICA.

(*a*) *The trophozoite.*

It should be stated in advance that up to the year 1908, when Bensen first questioned the identity of the human and the animal forms of Giardia, those studies which dealt with the morphology of the organism in greater detail, like that of Grassi and Schewiakoff (1888), Metzner (1901) and Wenyon (1907), had reference to animal forms exclusively, and the writers evidently assumed that these were identical with the human form.

Our knowledge of the structure of the human type, up to the year 1908, was based entirely upon relatively inadequate studies of the organism on the part of various clinicians. From these it was apparent that *Giardia enterica* was without a doubt closely related to the organisms described by Grassi and Schewiakoff, Metzner and Wenyon. No detailed description based on more modern methods of investigation was given, however, until 1908, in which year Bohne and v. Prowazek published the first account of the structure of the

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[*] In a recent contribution entitled *Giardia-Lamblia* by F. Reuling and E. Rodenwaldt (Arch. f. Protist., 1921, XLII, 337–345) the writers discuss the validity of suppressing the genus name *Lamblia,* as being synonymous with *Giardia.* They conclude that there is no sufficient basis for regarding the two as separate genera, but that it might be justifiable to create two subgenera, under the genus *Giardia,* viz., Subgenus *Giardia s. str.* and subgenus *Lamblia.*

To sum up, then, the results from both guinea pigs indicate that the partial or complete disappearance of the trypanosomes from the blood is associated with their actual destruction by the host, rather than with any retardation or inhibition of their reproductive rate.

These two guinea pig infections bring out a point which is often observed in the laboratory, namely, that the death of the guinea pigs is apparently not dependent upon the actual number of organisms per cmm. of blood. The death of the guinea pig may occur when the number of the organisms is at the highest point (pig 721) or it may occur when the organisms are comparatively very few in number and are actually disappearing from the blood (pig 719). The same condition of affairs, as we have already pointed out, holds for the dog, but it is not true for the rat or mouse. In the last two animals, death always occurs when the trypanosomes are most plentiful in the blood, and apparently never takes place until the host is suffering from an exceedingly high grade of blood infection.

THE COEFFICIENT OF VARIATION FOR SIZE AS A COMPARATIVE MEASURE OF THE RATE OF REPRODUCTION.

In the *Introduction* we gave a short account of the fundamental basis for using the coefficient of variation for some factor involving size as a comparative measure of the rate of reproduction or growth. We also pointed out its obvious advantage, viz., that it enabled us to estimate the rate of reproduction, even though most of the individuals resulting from this reproduction were killed. In other words, it is independent of numbers as long as we can measure enough individuals to form a sample of suitable size for statistical analysis. In presenting the preceding data we have assumed that it was a valid method for the estimation of the rate of reproduction of the trypanosomes. The establishment of its validity, however, is so fundamental from the standpoint of the present work that it seems advisable to discuss it again in this place. Such a discussion can be taken up under several heads.

A. Rationale of its use. As has already been pointed out, it is perfectly clear from an *a priori* standpoint that reproduction with its production of young forms and consequent series of growth stages must necessarily increase the coefficient of variation for size. Furthermore, it is also evident that this increase in variability is more or less proportional to the rate of the production of young forms, or, in other words, to the rate of reproduction. This reasoning should be valid given time. Taking these facts into consideration we have been able to differentiate between them by making frequent determinations of the number of organisms per cmm. of blood and of the coefficient of variation for total length, which gives us a comparative measure of the rate of reproduction of the trypanosomes, regardless of the number destroyed in consequence of resistance. The rationale of this general procedure is given on page 267 and a discussion of the validity of the coefficient of variation for total length as a comparative measure of the rate of reproduction is given on page 305.

Before leaving the question of the coefficient of variation for total length, it is well to call attention to the fact that in our tables we have given not only the actual coefficient, but the mean length and standard deviation from which the coefficient of variation was calculated. Incidentally, these tables contain some very valuable data in regard to the effect on total length, of growing the same strain of trypanosomes in different hosts. We will not consider this question in the present study, but will reserve it for a later paper.

Turning now to a consideration of the resistance which a host offers toward a trypanosome infection after the parasites have established themselves, we find that three different conditions have been brought out by the preceding experiments. In the first place, the highest grade of resistance was found in the non-pathogenic *T. lewisi* in the rat. Here the host builds up an ever increasing resistance which retards the rate of reproduction of the trypanosomes. This resistance is so pronounced that, during the last (or "adult") stage of the infection, the parasites exist in the blood in the so-called "adult" condition, without exhibiting any signs of reproduction or growth. We also find in this case that just as the resistance against the rate of reproduction is reaching its climax (viz., 10th day in rat 105), the host suddenly develops a resistance which destroys a majority of the organisms in the blood. The remaining parasites live in the blood for varying lengths of time (sometimes several months) until they are suddenly destroyed as the result of the final resistance which the host builds up against them. After their disappearance from the blood, the host is immune to another infection of *T. lewisi* (see Laveran and Mesnil, 1912). In the second place, we find just the opposite condition of affairs in most infections of rats and mice due to pathogenic trypanosomes. Here (with the exception of long infections, such as rat 709 and rat 729) we can find no evidence that any resistance is built up either toward the rate of reproduction or toward the destruction of the parasites, after they are formed. Once the trypanosomes establish themselves in the host, they reproduce and live in the blood unmolested. In some cases, indeed, there is evidence that they reproduce more rapidly as the infection progresses (rats 619 and 703). In the third place, we find in the relapsing type of infection (pathogenic trypanosomes in the dog and guinea pig and *T. equinum* in the rat) that no resistance is built up which retards the rate of reproduction of the trypanosomes, but a resistance does arise which destroys a large number of the parasites after they are formed.

The fact that resistance in the non-pathogenic *T. lewisi* produces two different effects whereas resistance in pathogenic species (if present at all) produces only one of these effects indicates that in the non-pathogenic forms there is either an entirely different type of mechanism of resistance, or that there is a different type of mechanism superimposed on the mechanism found in the pathogenic forms. It is interesting to note that Miss Robertson (1912) has brought forward considerable evidence to show that in *T. gambiense* in the monkey there is an endogenous cycle, in which phases of active reproduction alternate with phases of little or no reproduction. If this be true, it would indicate that this form in the monkey encounters much the same type of resistance as *T. lewisi* in the rat, except that in the latter case, when reproduction once ceases it never starts again. Miss Robertson's results indicate that *T. gambiense* in the monkey encounters a resistance midway between the type we have described in *T. lewisi* in the rat and the pathogenic species in the guinea pig. Such a result would not be at all surprising, and with the growing evidence that *T. gambiense* can exist in certain mammals as a non-pathogenic parasite it may be possible to find a host in which this trypanosome encounters the same type of resistance as *T. lewisi* in the rat.

In considering the resistance encountered by the pathogenic trypanosomes, the question immediately arises what effect the development of a resistance has on the time of death of the host. In the case of these trypanosomes in the rat we found one case (*T. rhodesiense* in rat 709) which strongly suggested that the development of a resistance tending to destroy the parasites actually prolonged the life of the host. In other words, there seems to be a definite connection in rats, and probably mice, between the number of organisms in the blood and the time of death of the host. This correlation does not, however, seem to hold good when the same trypanosomes are grown in the dog and guinea pig. In our two experiments on dogs, both animals died when the number of trypanosomes in the blood was very much less than it was at the peak of the infection. Of the two guinea pigs one died at a time when the number of trypanosomes in the blood was at its maximum height, whereas the other died when they were comparatively few in number. Of course, we can not say that the development of a resistance in the latter hosts did not lengthen the life of the animal. The results themselves, however, do not reveal any obvious connection between the two factors as was suggested in the case of *T. rhodesiense* in rat 709.

In our preceding discussion we have assumed that a disappearance of the trypanosomes from the peripheral blood indicates that they have been actually destroyed by the host and not that they have migrated into the tissues. As has already been pointed out, this seems to be a safe assumption to make, because Levaditi and McIntosh (1910) have demonstrated the presence of a trypanolytic antibody after the crises in such infections. Then, too, if the disappearance from the blood were to be explained by a migration into the tissues, there should be a great many more parasites in the tissues during the period of low blood infection. At the present time, there is not the slightest evidence that this is true. The same conclusion was reached by Miss Robertson (1912), although on slightly different premises. Of course, had we worked with *T. cruzi*, the condition of affairs would have been different, because this trypanosome shows an entirely different behavior and method of reproduction than do any of the other trypanosomes. In fact, the most typical form of this parasite, in the vertebrate host, is an almost true tissue-inhabiting Leishmania.

We feel that, in the present work, we have been able to introduce a new method of attack on some of the problems of resistance. This method, however, deals with the *effect* of the resistance, and not with its *mechanism*. What might constitute the most probable explanation of the effects which we have observed would necessitate a lengthy discussion. This, however, had best wait until we can do some actual experimental work on the question. We will point out, however, that the two different types of resistance in *T. lewisi* may be connected with the same fundamental mechanism.

In the future, we hope to continue the study of resistance and extend our method, if possible, to other infections. It is obvious that it can not be used unless accurate measurements of the size of the parasites can be made. This can be done for a number of protozoa, but it is impracticable for most of the bacteria. It is possible, however, that the spirochetes will lend themselves to this method of attack.

GENERAL SUMMARY.

1. The conclusions reached in this paper are based on the study of 13 experimental infections with trypanosomes, divided as follows: *T. lewisi* in the rat (2), *T. equiperdum* in the rat (1), *T. equinum* in the rat (1), *T. brucei* in the dog (1), mouse (1), and guinea pig (1), and *T. rhodesiense* in the rat (3), mouse (1), dog (1), and guinea pig (1). In the study of these infections determinations of the number of trypanosomes per cmm. of blood were made on 125 days and drawings, measurements, and computations of statistical constants were made for 114 sets of 100 trypanosomes each.

2. The resistance which a host builds up toward a trypanosome infection may do one or both of two things: (*a*) it may retard the rate of reproduction of the parasites, or (*b*) it may destroy the parasites after they are formed.

3. These two conditions can be differentiated by ascertaining: (*a*) the changes in the number of parasites per cmm. of blood, supplemented by (*b*) the changes in the coefficient of variation for total length of the trypanosomes, which gives a comparative measure of their rate of reproduction, irrespective of the number destroyed in consequence of the resistance. These two determinations differentiate between the two types of resistance because:

The number of trypanosomes per cmm. of blood = the number produced by reproduction of the parasites — the number destroyed in consequence of the resistance.

4. The validity of the coefficient of variation for total length, as a measure of the rate of reproduction of trypanosomes, is discussed in detail on page 305. Its great value lies in the fact that it measures the rate of reproduction irrespective of the number destroyed by the resistance.

5. Three conditions have been found in regard to the resistance to trypanosome infections.

(*a*) In the non-pathogenic *T. lewisi* in the rat, there is a resistance built up which is directed both toward a retardation of the rate of

In Plate XXIII, Fig. 4, finally the writer has reproduced one of a pair of dividing organisms, pictured by Wenyon and O'Connor in their monograph on "Human Intestinal Protozoa in the Near East" (1917) which will give an idea of the structure of the organism as they conceived it to be, though they give no detailed description.

(*b*) *The encysted form.*

While both Lambl and Grassi probably saw the cysts of the human Giardia in their preparations, they evidently did not recognize their relation to the vegetative form. The credit for having established this for the first time belongs to Perroncito (1887). He states that he found that the vegetative forms predominated in the stools of his patients when there was diarrhoea, while cysts only were found when there was constipation. He does not give a detailed description of the cysts and evidently did not study any stained specimens. He merely states that "the little bodies which are round or oval show distinctly cellular contents, amongst which a flagellum can be discerned." As measurements he gives 17 μ for the length and 10 to 12 μ for the breadth. He claims that in soft stools the organisms may be found in various stages of encystment. Regarding the significance of the process of encystment he concludes that the cysts are destined to keep the parasites alive and to bring about their dissemination.

The cysts have subsequently been mentioned by practically all observers who have come in contact with cases of giardiasis, but no attempt to study their morphology in detail was undertaken until Rodenwaldt did so in 1912.

Salomon (1899), however, noted the presence of 1 to 3 slightly curved lines and near their origin small nuclear structures in addition to certain sickle shaped markings.

v. Prowazek (1908) recognized that within the cyst the organism undergoes binary division, the resulting individuals subsequently becoming twisted so as to lie in opposite directions to each other. He and Schaudinn are responsible for the doctrine that a copulation

under this in their forward course. This conception of the course of the peristomal fibril is diagrammatically represented in an illustration which accompanies the article. He has evidently abandoned the view that this fibril starts from an outer pair of basal granules. In this respect the writer agrees with Rodenwaldt, but he would emphasize that he has never seen any appearances which would suggest that in adult organisms at any rate the fibril in question is continuous in its posterior half and that the axostyles and postero-lateral flagella pass beneath it.

process takes place in some of the cysts (copulation cysts). v. Prowazek thus states "Occasionally it was noted that two individuals had come into apposition and had secreted a cyst wall." The four nuclei divided once more and the resultant products emerged from the peristomal border which was visible within the cyst in the form of two strongly curved lines. Subsequent changes could not be followed with certainty. Bensen's account of cyst formation (1908) was evidently influenced by that of v. Prowazek and Schaudinn. He states the following: "Cyst formation proceeds in such manner that two individuals come into ventral apposition and then encyst. Within the cyst they loose their flagella and a union of the nuclei results. Further changes have, however, not been observed." He regarded these copulation cysts with two nuclei as characteristic of the human form. Noc (1909) states that he was unable to observe either copulation or autogamy in the cysts. He points out that nuclear division within the cyst is not necessarily limited to the binary type, but may terminate in multiple division with a corresponding division of the cytoplasm. His subsequent account of what occurs is, however, somewhat confused and his illustrations are unfortunately so crude that they do not aid in the elucidation of his meaning. The "corps énigmatique" according to Noc undergoes longitudinal cleavage within the cyst and "one asks oneself whether the resulting chromatin filaments may not serve to regenerate the posterior flagella of the daughter giardias within the cyst."

Rodenwaldt (1912), as I have stated, has given the first detailed account of the structure of the human cysts. Concerning the existence of copulation and autogamy cysts, as described by earlier writers, he thinks that such an assumption "seems somewhat audacious in view of the imperfect illustrations which have been furnished." He himself is willing to admit only "that he has evidence of division into two." "Some of the earlier stages of division," as he has seen them, "resemble the pictures of autogamy cysts of various observers and some of the more advanced stages the so-called copulation cysts." An outline of Rodenwaldt's own findings follows: Cyst formation is preceded by the appearance of a dense layer of fine granules beneath the arched dorsal surface; at the same time this latter increases in size. The dorsal portion of the animal extends ventrally over the sides, with the result that the contents of the peristome become folded in more and more. Rodenwaldt assumes that the cyst wall is formed from the granules just mentioned, because they disappear completely with the completion of the process of encystment. Inside of the denser outer covering there is in every cyst a broad, light, non-staining layer which probably represents a soft plasmatic covering of the organism within. In young cysts one can clearly see two nuclei and between them the arrangement of the basal granules with their fibrillar apparatus, with the exception of the periostomal fibril which has become separated and is curled into a dense skein whose individual loops, when viewed in a single plane, only at times appear in the form of separate arched or clasp-like bodies. This entire mass tends to sag to the posterior pole. The decussation of the anterior flagella is at this stage still distinct while the other flagella are located toward the other pole and often cross each other. The "eigenartige Körper" assumes a fore and aft position and seems to divide into two long compact rods. Rodenwaldt suggests that these may possibly serve to direct the two new nuclei which result from the division of the original pair, toward the posterior pole, where they are later found. After that phase has been reached the "eigenartige Körper" disappears and reappears only when the young animal has matured to a certain stage (see Plate XXIV, Figs. 1–4).

Immediately following encystment the division of the nuclei begins, during which process the chromatin breaks up into a larger number of fragments than were present originally. Rodenwaldt states that he was unable to count the exact number. During these stages of the process the basal granules of each side (it will be remembered that Rodenwaldt pictures and describes four) move together so closely that each pair appears like a single granule. Cysts of this type are the most common and when seen from the side have no doubt given rise to the idea of copulating organisms.

As the new set of nuclei moves to the opposite pole they take with them a set of flagella. During this process the cyst assumes a roundish form. After the migration has been completed there is a return to the oval form. At the same time the dense skein of the perinuclear fibril splits into two and the two skeins move apart (Plate XXIV, Figs. 1–4). The actual process of excystment Rodenwaldt was not able to observe. Occasionally he met with cysts which were evidently degenerating and in which larger masses of chromatin could be discerned together with remnants of nuclei. Such appearances simulate pictures which may have suggested the idea of schizogony to Noc.

ORIGINAL OBSERVATIONS.

Material and methods.

My own studies, so far as the vegetative forms are concerned, are based upon material which was freshly obtained (*a*) from three individuals who had probably acquired the infestation in Maryland, (*b*) from one South Carolina case; (*c*) from a United States soldier, who may have acquired the infestation in France (where the preparations were made). Cyst material was obtained fresh from three Maryland cases, from an individual living in Washington, D. C., and from one case who had acquired the infestation in Brazil. In addition cyst material was also available from two Chinese cases. This had been preserved when fresh in 4 per cent. formalin, and was used for comparative studies of a corroborative nature only, and not for measurements.*

Permanent preparations were made by fixing wet smears in Schaudinn's solution (at a temperature of about 50°–60° C.), and subsequently staining in Heidenhain's haematoxylin, using iron alum in 4 per cent. and 2 per cent. aqueous solution as mordant and differentiating agents respectively. For the study of the flagella eosin-methylene azure mixtures were found most useful, and occasionally Mann's eosin-methyl blue mixture was also employed.

PLAN OF STUDY.

From the survey of what has already been done on the morphological study of *Giardia enterica* it is clear that an attempt on our part to furnish a description which can be used for comparative purposes will have to be based upon a critical analysis of the findings of these earlier investigators, and a comparison of their results with my own. For convenience sake we shall study the organism, both in its vegetative and its encysted form under the following headings:

1. General morphology.
    (*a*) Form and general structure.
    (*b*) Supporting structures.
    (*c*) Neuro-motor apparatus.
        (*α*) Nuclei and their relation to the motor apparatus.
        (*β*) Origin and course of the flagella.
    (*d*) The so-called parabasal body.
2. Biometry.

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* The writer is indebted to Dr. W. C. Boeck for the material from the French case and the individual living in Washington, D. C.

THE TROPHOZOITE.

Form and general structure.—As seen *en face* the young and fully grown trophozoites appear roughly pyriform in shape, the rounded wider portion corresponding to the anterior end and the narrow portion to the posterior end. The greater portion of the anterior end is occupied by a depressed area which represents the so-called sucking cup in which two nuclei can be clearly distinguished. These structures are convenient anatomical landmarks by which we can fix the lines of maximal width, etc., and the location of the various organelles. In most specimens the line of maximal width drawn at right angles to the longitudinal axis passes through the posterior poles of the nuclei. If we denote the entire length of the organism by the figure 100, the distance from the anterior pole of the body to the line of maximal width, as just defined, represents approximately 23.5 per cent. of the total. From the extreme outer points of this line of greatest width the lateral lines approximately maintain the same distance from the longitudinal axis for about 14.5 per cent. of the total length. Thereafter the lines gradually converge; the remainder of the length corresponding to about 26 per cent. of the total. In some specimens the posterior end becomes very much narrowed, the lateral lines running almost parallel for a distance of about 2 micra, while in others, and these constitute the majority, there is a gradual convergence toward the caudal termination (see Text figure 1).

In addition to the gracefully elongate forms which may be viewed as typical of the vegetative type one now and then encounters in nearly every slide organisms whose contours tend toward the oval and in which no marked attenuation of the tail end exists. These forms the writer is inclined to view as old forms which are preparing for encystment, without wishing to suggest, however, that only old forms can and do encyst.

When viewed from the side the organism presents a slightly upturned attenuated head tip. Immediately behind this appears a suggestion of a slight depression from which point off the dorsal surface becomes markedly arched, the highest point being reached about the middle of the posterior half of the body; there is then an abrupt drop to very near the end of the tail, and a final brief convergence with the latter.

An examination of the ventral surface* shows a cup-like depression occupying almost the entire anterior portion of the body and extending nearly to an imaginary line cutting the longitudinal axis at right angles, at a point about half way between the anterior end and the tip of the tail. The cup is roughly kidney shaped, its long axis being at right angles to the long axis of the body. At its hilum we meet with the anterior portion of a diamond shaped depressed area which is bordered at each side of its anterior half by a triangular shield-like ventral incurvation of the dorsal surface of the animal (see Text figure 1). The diamond shaped area just mentioned evidently becomes continuous with the cytostomal depression, by way of the hilum which latter region I would propose to name the cystostomal vestibule.

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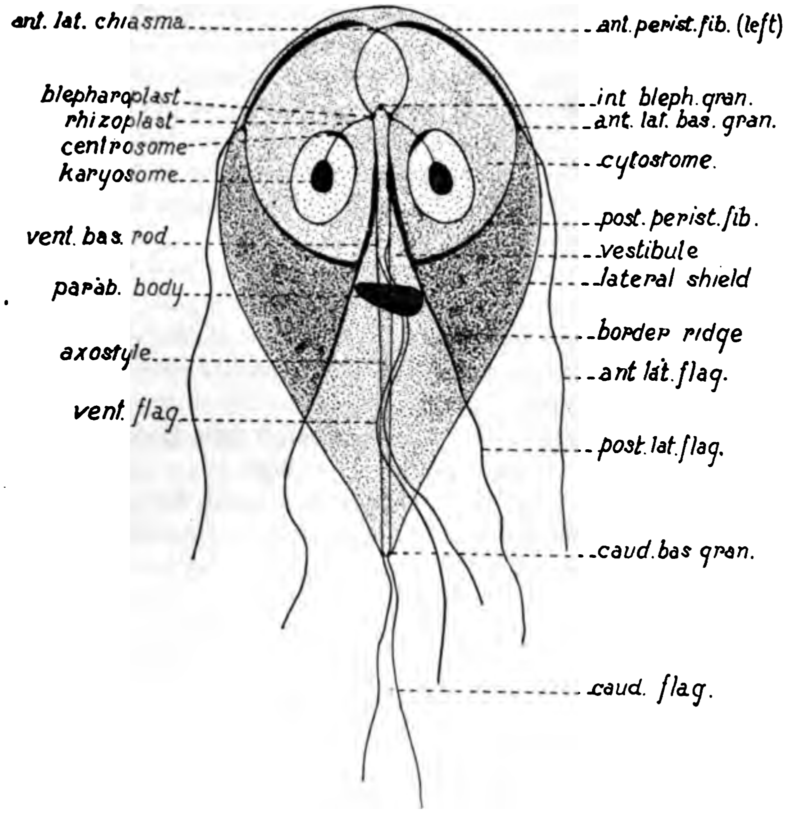
* Bearing in mind that the so-called parabasal body is located dorsally to the axostyles it is easy to determine whether we are looking at the dorsal or ventral



TEXT FIGURE 1.

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surface by focussing down slowly and noting whether the plane of the parabasal body or that of the axostyles appears first.

Lying in the depth of the cytostome apparently there are the two nuclei with which are connected, in a manner presently to be described, the four pairs of flagella. The two anterior flagella—the antero-laterals—become free at a level corresponding closely to a line drawn through the anterior poles of the nuclei. The postero-laterals emerge laterally a little more than half way between these points and the tail end of the animal. The caudals emerge from the extreme posterior end. The fourth pair, the ventrals, originate apparently in the cytostomal vestibule in a manner which will be described below, and seem to become free at once. The "mysterious body," or parabasal body, as it is now called, lies a little posterior to the vestibule, within the cytoplasm between the ventral and dorsal surfaces, as may be seen in side views of the organism.

The cytoplasm in stained preparations presents an alveolar rather than a granular appearance; on the surface it has become concentrated into a fairly resistant homogeneous periplastic layer. Possibly derived from this latter through a further process of condensation, are the following structures:

Supporting structures.—It will be recalled that Rodenwaldt conceived the cytostome to be bordered by a single stout fibril which he thought originated in the cytostome from a basal granule and then ran around the entire margin to end in a corresponding granule on the other side (see Plate XXIII, Fig. 3). This conception the writer believes to be untenable. In carefully destained specimens it can be seen distinctly that two deeply staining fibrils run along the anterior margin of the cytostome from the points where the antero-lateral flagella become free to near the middle of the anterior arch of the border of the cup. At this point however, there is a definite gap over which the two fibrils do not pass. The idea that the course of the fibril is continuous at this point can only have arisen from the observation of insufficiently destained specimens, or of specimens in which the cytostomal margin had contracted at the time of fixation. This latter possibility will readily be appreciated, if it is borne in mind that during the life of the organism the cytostomal aperture is undergoing active expansile and contractile movements, while the parasite is not attached to a cell. Some specimens will naturally be fixed while the aperture is contracted. But even though specimens be present in which this is expanded it is very easy to overlook the little break in the arch and particularly so when the process of destaining has not been carried far enough.

in the past, and up to the time of the great war the majority of the papers dealing with the subject were essentially devoted to the recital of isolated cases or small groups of cases. The infestation was largely looked upon as a medical curiosity and as of negligible importance. Very little was known of the geographical distribution of the parasite, of the extent of its occurrence in those countries where it had been observed, of its pathogenicity, of the mode of infection and hence of its control. Up to the present time (1921) an adequate morphological basis for comparison of the human type with the forms found in various animals did not even exist. A considerable amount of interest in these various questions was, however, evoked by experiences gained in the course of the war. It was then found that the infestation of man was much more common than was formerly believed, that many individuals harbor the parasite without evidence of any harmful effects, it is true, but that in many others obstinate diarrhoea exists which can not be attributed to any other cause. But even so, we are evidently only beginning to appreciate that giardia is a parasite which is worthy of study. During the past two years the writer has devoted a considerable part of his time to the investigation of some of these problems, and it is intended to present the results of his investigations in a series of papers, of which this is the first.

THE PROBLEM.

The present paper is intended as a contribution to the question whether human giardiasis is of rodent origin. To determine this point it is essential to develop a picture of the parasite, as it occurs in man, which may serve for comparison with the forms which occur in the animals under consideration. Up to the present time such a standard has not been available. There has been a gradual evolution of the final picture, as will appear from a survey of the work done in the past, but even the latest account, given in 1912, leaves many points undetermined. Before proceeding to this survey a few words regarding the taxonomics of the organism may not be out of place.

TAXONOMICS.

The organism which was discovered by Lambl in 1859 was thought by this observer to belong to the genus Cercomonas. From its habitat he termed it *Cercomonas intestinalis*. Grassi (1879), who apparently was in ignorance of Lambl's discovery, described what was evidently an organism belonging to the same genus, but occurring in various the cytostome. This may be observed in animal material containing both living specimens and epithelial cells of the intestinal tract. It will then be seen that in trying to attach itself the animal first hooks the anterior arch over the top of the cell and then pulls itself into position. The rigidity of the two posterior arches is evidently enhanced by the rotundity of the two lateral shields, which in turn are stiffened along their inner margin by the so-called intracytoplasmic portion of the postero-lateral flagella. An examination of these regions will show that from the points of the lateral emergence of the exceedingly delicate flagella a line of increasing thickness, as measured by the degree of staining, on each side passes along the inner border of the triangular shields, and entering the vestibule runs forward to a level with the posterior end of the anterior third of the nuclei, the stoutest portion being the pre- and intra-vestibular portion. At the points mentioned these lines which within the vestibule run a nearly parallel course seem to stop as such, but are continued forward for a short distance by two much finer fibrils. I have gained the impression that what we see of the so-called intracytoplasmic prolongation of the postero-lateral flagella no more represents such structures than do the anterior peristomal fibrils represent the antero-lateral flagella. The diamond-shaped depressed portion which extends to the tail from the vestibule is, in my opinion, demarcated from the lateral shields by a wall which rises in height from the posterior angle of the shields and reaches its highest portion within the vestibule at a point from which other structures presently to be described evidently dip down to the bottom of the cytostome. I am not prepared to positively assert that a separate detachable fibril runs along this border, for it is quite possible that there is only a thickening of the cuticle, but certain structures that one meets in encysted forms suggest this possibility. For convenience sake I would propose to call the lines in question the border ridges of the lateral shield lines.

As regards Rodenwaldt's marginal fibril—Randfibrille—which he conceives as laterally bordering the posterior portion of the body of the animal, I have not been able to satisfy my mind that such a fibril exists, while a cuticular thickening may often be observed running along the outer margin of the lateral shields and even extending to near the end of the tail. In specimens that are apparently about to encyst this line is particularly well marked; it seems to be continuous with the head arch and in some specimens curves around to the other side, crossing over the posterior surface of the tail. Such specimens look as though the animal were covered with an oval shell, like a turtle.

Rodenwaldt, who maintains the existence of two axostyles running to the caudal end of the body from a pair of basal granules lying in the depth of the cytostome between and a little anterior to the anterior poles of the nuclei, regards these as the "Hauptstützfibrillen," the principal supporting fibrils of the animal. The writer does not share this view. The writer, like Rodenwaldt, maintains the existence of two "axostyles," but in his estimation these are too delicate to serve as supporting structures, and he looks upon them purely as true intracytoplasmic continuations of the caudal flagella. In some specimens, particularly such as have not been sufficiently destained, the caudal halves of these structures are seen in close apposition and may then suggest a single body, but from the level of the parabasal body forward into the vestibule one can *always* see two fine lines which pursue a parallel course, the interval between which is always clear and distinct. In most thoroughly destained specimens there is no difficulty in distinguishing the course of these two lines posteriorly where they end not in one, but in two basal granules. (We shall revert to this question a little later on when discussing the neuromotor apparatus in detail.)

To recapitulate, the writer would look upon the periplast of the organism as an important supporting structure in itself. This is thickened along the inner and possibly the outer border of the lateral shields, and to some extent along the lateral border of the tail. Derived from it, no doubt, are the four separate fibrils which run along the border of the cytostome, viz., the two anterior and the two posterior peristomal fibrils. These are separate and apart from the neuromotor apparatus, which we shall now consider.

The neuro-motor apparatus.—The neuro-motor apparatus of the organism is composed of two nuclei, a number of centrally located basal granules connecting with the nuclei by means of rhizoplasts, four pairs of flagella, and peripherally located basal granules (see Text figure 1).

The two nuclei are seen in the depth of the cytostome. Their anterior poles converge slightly toward the longitudinal axis of the animal. Seen *en face,* as well as from the side, they are oval in form. The chromatin in the resting nucleus is gathered into a round or oval karyosome which is united to a fairly large somewhat crescentic appearing mass of chromatin, which is apparently attached to the anterior pole of the nuclear membrane by means of a rhizoplast and which may be viewed as a centrosome. More rarely a little mass of chromatin is seen at the posterior pole or in the lateral wall of the nuclear membrane. The membrane is always distinct. A little anterior to the level of the anterior poles of the nuclei and between the two are two deeply staining granules which in some specimens can be seen to be united by rhizoplasts to the centrosome of the corresponding nuclei. They are, moreover, united with one another by a little arched commissure which carries a small granule in the center and which I would term the interblepharoplastic granule. The two outer granules are viewed as blepharoplasts. Arching out from them and running forward to cross near or in the little central gap, between the anterior peristomal fibrils, are the anterior flagella. At the point of crossing—the chiasm—a tiny granule can often be discerned. From this point on their course is obscured by the peristomal fibrils. They then emerge laterally where the latter end, and at times a little granule may be seen at the point of exit from the body of the animal. The free flagella are very delicate and can usually not be seen distinctly throughout their course in haematoxylin preparations. With Giemsa's stain, however, they can be brought out very well, and in such specimens it will be seen that they are fully as long as or longer than the entire body of the parasite. Running backward from the blepharoplasts for a distance corresponding to about the level of the posterior end of the anterior third of the nuclei are two fine parallel lines. At the level just mentioned these apparently merge with the heavily staining stout anterior endings of the border ridges of the lateral shields. From the points where these begin to diverge two fine lines run posteriorly, following a parallel course as far as the anterior level of the parabasal body, after which they gradually come closer together and finally terminate in two caudal granules, which are separated by a very small interval and from which the two caudal flagella issue. The latter are approximately as long as the entire animal. In this connection it may be mentioned that in some specimens the tail is bifid, with a granule at each end, and that in others the flagella become free a little distance before the end of the tail is reached. For the sake of convenience we may call the intracytoplasmic prolongations of the caudal flagella axostyles, but, as I have stated already, these structures seem to be too delicate to serve as supporting organs. It is possible that in the beginning of their course (reckoning from the tail end forward) they may be running along a shallow and narrow groove as far as the level of the parabasal body. This might account for the fact that in insufficiently destained specimens this portion seems to stain somewhat diffusely, and hence suggests the existence of a single axostyle. But as I have said before, the lines running forward from the level of the parabasal body are always distinctly separated with no intervening staining material of the same color whatever, and in large numbers of specimens the separate course of these fibrils can be traced to the tail without any difficulty. Kofoid and his pupils describe and picture but one axostyle as the normal in resting specimens of the animal forms which they studied in California, viz., *Giardia muris* and *Gardia microti*. According to these investigators, the axostyle starts from a single caudal basal granule and runs forward, becoming wider as it proceeds, until its termination is reached in the form of the little interblepharoplastic commissure mentioned above. When division is to take place the axostyle, according to Kofoid, splits at its broad end, with the simultaneous formation of two pairs of blepharoplasts, and gradually the split is supposed to extend to the caudal end. I have never seen any appearances suggesting such a process of division in my human material. At first I was inclined to believe that my findings were due simply to a different interpretation from that of Kofoid, but after having studied thousands of specimens I can not help but believe that my interpretation expresses the true state of affairs. Kofoid in his dividing forms pictures the commissure as broken. Whenever I have seen anything suggestive of the presence of four blepharoplasts the commissure was still intact and it appeared as though the second pair were located dorsal to the first pair. I may add that a careful study has convinced me that there is no relation between the stage of chromatinic division within the nuclei and the appearance of the two axostyles, as I have described it. As additional evidence of the normal presence of two axostyles, I may briefly record the following observation, which will be published in greater detail at another place: In the feces of one of my patients large numbers of organisms were encountered which had evidently just emerged from the cysts. In so far as it was possible to obtain a clear picture of the structures under consideration, two axostyles were seen in these forms also. Whether we have a right to call these fibrils axostyles seems rather doubtful to the writer and he would suggest that the term intracystoplasmic continuations of the caudal flagella would be more correct.

The ventral flagella apparently originate from slightly elongate basal granules or rods which seem to be attached to the axostyles about where these enter the vestibula, a little anterior to the parabasal body. They become free at once and in the living animal are engaged in a corkscrew-like motion which continues long after the lateral and caudal flagella have become quiescent. Their motion, moreover, continues while the animal is attached to a cell. They constitute its principal motor apparatus, while the other flagella execute only sluggish movements, by which the animal would be propelled only in a very indifferent manner. The ventral flagella are usually not distinct in iron-haematoxylin specimens, but may be brought prominently into view by staining with a Romanowsky mixture. Their length is somewhat difficult to measure, but apparently they extend to the end of the tail.

The postero-lateral flagella emerge at the tips of the lateral shields, which points lie a little more than half way between the caudal tip and the points of emergence of the antero-lateral flagella. v. Prowazek and Rodenwaldt both look upon the dark line which constitutes the inner border of the lateral shields as intracystoplasmic portions of these postero-lateral flagella. For reasons which I have already mentioned, I do not share this view, and it is my belief that the flagella in their course forward run along or beneath this heavy lateral line. They probably join the so-called axostyles in their intravestibular course, and conjointly with these reach the blepharoplasts. The extracytoplasmic portion of the postero-lateral flagella can be well brought out with a Romanowsky mixture and is about as long or longer than the entire body of the animal.

The "parabasal body."—This structure in the human type of Giardia usually presents a club-shaped or a heavy comma-like appearance, and in iron-haematoxylin specimens is stained black. Lateral views show that it is located dorsally to the "axostyles." Its longitudinal axis lies obliquely to the longitudinal axis of the organism, the narrow end slanting toward the posterior pole. Occasionally one gets the impression as though the structure were split longitudinally. A noteworthy fact is the frequent absence of this body. The writer has carefully studied the question whether any relation exists between the presence or absence of this body and the phase of the nuclei as regards evidence of beginning division, and also in reference to the question whether the axostyles appear single or double throughout their course. Many hundred specimens were examined from this point of view, but as the data presented in the accompanying table show, no relation between these factors could be discovered.

Nuclear phase.	Parabasal body.	Appearance of axostyles.
1. Quiescent........	Full size	Distinctly double to caudal termination.
2. "	Almost gone	" " " " "
3. "	Absent	" " " " "
4. Eight granules in parallel rows...	Full size	" " " " "
5. Eight granules in parallel rows...	Absent	" " " " "
6. Late prophase....	"	" " " " "
7. Late prophase....	Full size	" " " " "
8. Late anaphase....	Full size	" " " " "
9. " "	Almost gone	" " " " "
10. Metaphase.......	Full size	" " " " "

Sometimes the body in question stains deeply, at other times scarcely at all. Sometimes its length equals or even exceeds that of the nucleus; at other times it is so small as to be scarcely discernible. The body is thus evidently not of a permanent nature, or perhaps it would be more appropriate to say the staining substance is not of a permanent nature. The fact, however, that when this staining substance is present it always seems to occupy the same position suggests the continued presence of a structure in which the staining substance is temporarily stored. Boeck (1919) has also come to this conclusion during his study of rodent forms. He states: "In about half of the flagellates the parabasal bodies appeared to be situated upon or in a definite area of the cytoplasm, limited by a membrane-like structure." This writer found that on staining with acid fuchsin and methyl green the bodies appeared red and the chromatin of the nuclei green, while in specimens stained with basic fuchsin the parabasals were not stained at all. With Wright's stain I found that the structures took the red in the same tone as did the nuclei and the flagella. With Mann's eosin-methyl blue mixture they were colored red, as were the karyosomes of the nuclei.

On staining Schaudinn preparations with Lugol's solution, Boeck found that a certain number showed a mahogany colored mass "filling all the parabasal-plasm," and he concludes that the function of the bodies is to secrete glycogen which is retained for subsequent use in the parabasal-plasm. While the writer agrees with Boeck that the contents of the parabasals *may* be composed of glycogen, he does not believe that the iodine test alone can be viewed as a criterion of the presence or absence of glycogen. It should be borne in mind that the so-called glycogen vacuoles of certain amoebae are not stained by iron-haematoxylin.

Both Kofoid and Boeck mention that on rare occasions in some of their animal material they have seen fibrils connecting the parabasal body with the dorsal side of the axostyle. I have studied lateral views with this in mind and on one occasion thought I could discern a tiny fibril coming from a parabasal, but I was unable to trace its course.

The fact that I have been able to clearly recognize these bodies in all the very small forms which I encountered on one occasion and to which I have repeatedly alluded as probably representing freshly excysted forms, unless we assume the unlikely alternative that the enormous number had resulted from multiple fission of free forms, represents additional evidence that their presence or absence has nothing to do with nuclear division.

It will have been noted that I have avoided the term parabasal body as much as possible in referring to this structure, as its acceptance would involve the recognition of its homology with the parabasal body of Devescovina of Janicki (1911) and with the chromatic basal rod of Trichomonas, as was first tentatively suggested by Alexeieff (1914), and as apparently is held by Kofoid and Christiansen. The writer does not believe that the evidence which has thus far been adduced to support this idea is sufficient, and in a later paper he expects to discuss this question in greater detail.

THE ENCYSTED FORM.

The majority of the Giardia cysts in the stained specimen present an oval outline. In addition apparently spherical forms will also be encountered. Whether or not these are spherical in reality may, however, be questioned. It is possible that the spherical appearance may be due to the fact that we look down upon the rounded ends of otherwise oval forms.

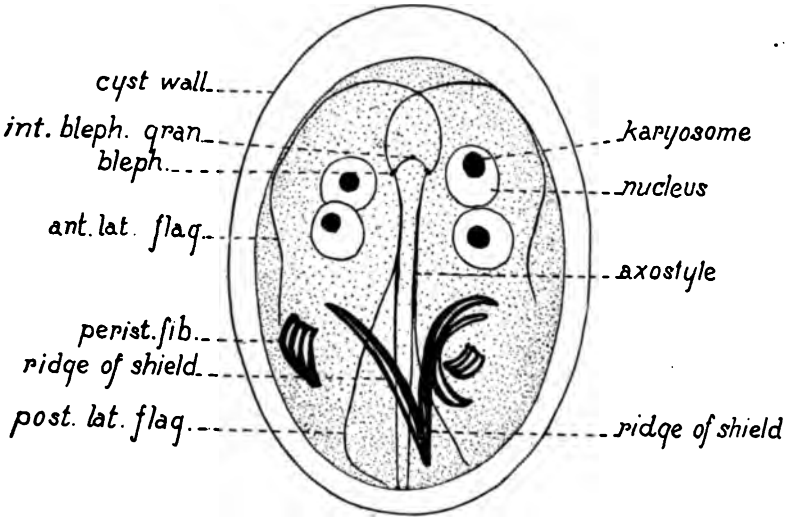
The cyst wall is apparently separated from the contents by a narrow layer of a non-staining substance. Within the cyst the organism first prepares for division and ultimately, no doubt, undergoes division, with the formation of two or more merozoites. The appearance of the contents will hence vary according to the stage of division and the number of merozoites that are being formed. An adequate mental picture of the process of division can, of course, only be formed from a study of a large number of specimens, not only from the same stool, but from stools obtained on different days, and even then it must be admitted that it is often exceedingly difficult, if not impossible, to say what this and that line in the cyst represents. It seems to be certain that at an early stage following encystment some of the structures lose their connection with the other organelles. This is true more particularly of some, if not of all, of the supporting structures. The consequence is that in most cysts one merely sees an apparently uniformly granular cytoplasm, and lying within this the nuclei and various more or less distinctly and deeply staining lines and fibrils.

In human cysts a binucleate condition is the exception. In the course of an analysis of 400 cysts from four different human hosts, 100 being studied in reference to this point in each case, the writer found that 96 per cent. were definitely qradrinucleate; the values per individual were 96, 94, 96 and 98 per cent. Binucleate cysts were rare, and a cyst containing more than 4 nuclei still rarer. I have never seen more than eight nuclei in a human cyst, and since practically all vegetative forms which one sees in the feces in the case of man are binucleate, one is forced to conclude that nuclear division must take place very quickly and very early after encystment has taken place. Preencysting forms, viz., such in which the outline of the vegetative form has become oval, are in the writer's experience all binucleate. In most specimens the four nuclei are located near one pole, usually two and two on different levels. In attempting to sketch such appearances it is hence necessary to show them as overlapping. In contradistinction to the vegetative forms the nuclei of the cysts are always spherical instead of ovoid. In their interior the chromatin will be found in various stages of division. With a sufficient amount of patience one can count eight chromosomes during one stage of the process. At one stage these arrange themselves in the form of an equatorial plate and subsequently combine in pairs, giving rise to four fairly coarse granules. The details of the process of division so far as the writer has followed them, seem to be the same as have been described for *Giardia muris* and *Giardia microti* by Kofoid and Christiansen and Boeck.

Lying between two of the nuclei one can see in most cysts two blepharoplasts still joined by their commissural fibril, at times still presenting the central granule; and running toward the posterior pole from these the two fine parallel lines that one sees in the vegetative forms. A short distance from the blepharoplasts one can then note a separation of the delicate postero-lateral flagella from the equally delicate so-called axostyles. In some specimens corresponding to this stage of development one can also see the anterior flagella, follow them after they have crossed, and observe them running down along the outer margin of the granular contents of the cyst. It is noteworthy that there are no lines in the cysts comparable to or in the position of either the anterior peristomal fibrils, or the posterior peristomal fibrils or the fibril-like structures which border the lateral shields. The ventral flagella I have at no time been able to see or identify in the cysts. At the end of each axostyle a basal granule is not infrequently discernible, and I have also seen granules which correspond in position to those from which the antero-lateral flagella emerge from the body.

In other specimens the blepharoplasts have apparently divided in a dorso-ventral direction, but even then the commissural fibril can yet be made out. A splitting of the fibrils running to the posterior end I have not been able to observe with certainty, at least not near their points of origin, though toward the far pole I have at times counted as many as eight delicate lines. More often the lines are so closely matted together that it is impossible to count them.

In addition to the structures that have been mentioned above one can see in practically every cyst certain deeply staining heavy lines. These may be divided into two groups. One group is represented by relatively short curved fibrils which on careful examination may be seen to be arranged in sets of four, each fibril terminating at one end at least, and the writer believes at both ends, in a fairly deeply staining granule. Sametimes one sees but a single set of these fibrils and at other times a double set. Rodenwaldt views these as liberated peristomal fibrils, but he evidently failed to recognize their number and separate existence and the terminal granules. The second set probably has resulted from the original set through longitudinal division. The second group of heavily staining fibrils is usually represented by two relatively long, slightly arched, fusiform, rod-like structures which are located in the posterior half of the cyst and flare out, the one to the right and the other to the left as shown in Text figure 2. Rodenwaldt suggests that these structures represent the "mysterious body" (parabasal) and that they may possibly serve to direct the two new nuclei, which result from the division of the two original structures, to the posterior pole. He adds that after that phase has been reached these bodies disappear, to reappear again only when the young animal has matured to a certain stage. The writer does not share this view, and prefers to leave the origin and nature of these rods undecided. His inclination is to view them as in some manner connected with or representing some of the supporting fibrils in so far as they are not represented by the smaller curved fibrils described above. Definitely opposed to the idea that they are derived from the parabasal body is the fact that with Mann's stain they do not take a red color as does the parabasal body of the trophozoite.



TEXT FIGURE 2.

Of the fate of the "mysterious body" we are in ignorance. If as Boeck suggests this structure merely represents a mass of reserve food material—glycogen—it would seem natural that this should be used up while such active changes are taking place, which involve the disorganization, division and reorganization of the organism.

In the very young forms to which I have referred above and which I am inclined to view as excysted forms the "mysterious body" could be made out, whenever the posterior end of the animal was sufficiently developed as to afford a clear view of the region which is occupied by this structure. One might accordingly question the disappearance of this body in the cyst. The writer can only say, however, that he has seen no structure which could be interpreted as such within the cyst, and it is certainly quite possible that the young organism may elaborate a reserve of food material as soon as it gets the opportunity. In some of the youngest excysted forms that I have seen there appeared to be attached to the ventral surface of the animal a delicate bud-like mass of granular cytoplasm. Whether this may represent a little store of food material with which the organism has started upon its career I do not know.

The fate of the parabasal body in the cysts I have not been able to determine. Boeck (1919) in describing his findings in the cysts of *Giardia microti* states that "In the cyst the bodies may be two or more in number and are greatly hypertrophied at the beginning of encystment. In the cyst the bodies are located in various regions, but always in the posterior part of the body. They often spread out like the tail of a comet or they may appear as conglomerations of a material, cloud-like in character, through the cytoplasm. Such was the condition of the parabasal bodies during multiple fission in *Giardia muris* (Kofoid and Christiansen, 1915)." From a study of Boeck's illustrations it is clear that he regards as parabasals those structures which the writer, as well as Rodenwaldt, looks upon as peristomal fibrils in various stages of division. Considering the normally small size of the parabasals it is difficult to conceive how the store of food material which they represent can possibly hypertrophy at a time when the animal can only break down what it has, but certainly not form more anew. Boeck pictures quite correctly the splitting up into fibrils with a granule at each end of these structures which at one phase of their development resemble the arched tail of a comet, but this splitting up would be a useless performance, if in the end they should merely disappear, and be it remembered that in his as well as in Kofoid's account of the structure of the cysts no reference is made to the fate or origin of the supporting structures.

I am quite willing to admit that I have not as yet been able to furnish the actual proof that the brush-like structures which Boeck looks upon as parabasals are in reality peristomal fibrils, but believe that the likelihood of such being the correct interpretation is greater than the probability of their representing hypertrophied parabasals. A food reserve certainly does not increase in quantity during starvation.

In conclusion I would state that I have never seen cysts derived from human beings in whom two fully developed organisms could be recognized within the cyst wall, which is so common in the case of the feces of meadow mice harboring *Giardia microti.* I have never been able to observe any further development in the cysts of *Giardia enterica* after they had been voided.

In the past it has been tacitly assumed that excystment does not occur in the digestive tract of the same individual, unless auto-infestation takes place. In the one case to which I have repeatedly referred in which what appeared to be immature forms were present in very large numbers, active movement was noted within many of the cysts in the freshly voided stools and demonstrated in the laboratory. This would suggest that the small forms which were present in the same stool were probably young excysted organisms and that excystment can actually occur within the digestive tract. Such an observation, however, is certainly exceptional, and it may be questioned whether it represents a normal process. Fully developed forms within the cyst wall were not seen in this case.

BIOMETRY.

In as much as earlier observers have given widely different measurements for the human type of Giardia, but without regard for the requirements of modern biometry, and in as much as the study of size of these low forms of life often plays an important rôle in inquiries regarding the existence of species, it seemed to the writer that a careful investigation of this phase of the problem was of paramount importance. The following factors were accordingly considered: In the study of the trophozoite: (*a*) the length of the organism; (*b*) its breadth; (*c*) the relation of length to breadth (BL/BB).; (*d*) the total area of the body; (*e*) the length of the nucleus; (*f*) the breadth of the nucleus; (*g*) the ratio of the length to the breadth of the nucleus (NL/NB); (*h*) the area of the two nuclei combined; (*i*) the relation of the nuclear area to the body area (BA/NA).

In the case of the encysted organism (*a*) the length of the cyst; (*b*) its breadth; and (*c*) the ratio between length and breadth (L/B) were similarly determined.

The material for the study of the trophozoite was obtained, as in the foregoing morphological study, from five different individuals which have been designated in the tables and curves as Maryland I, Maryland II, Maryland III, South Carolina and French respectively. The cyst material was also obtained from five different individuals which have been designated as Maryland I, Maryland III, and Maryland IV, District of Columbia and Brazil. Maryland I and III of this series were the same individuals as Maryland I and III of the trophozoite series. The number of specimens measured from each individual case is given in the first column of the tables and in the legends accompanying the curves. Each set of measurements, whether recorded in tabular or curve form, is accompanied by the corresponding composite value. In the table the range between the lowest and the highest values is given, together with the mean which has been calculated not on the basis of the individual means, but upon the measurements of the entire series. Measurements of size are expressed in micra, while measurements of area have been computed in square millimeters and correspond to camera lucida drawings of a magnification of 1600 diameters. The ratios expressing the relation of length to breadth were obtained by dividing the length by the breadth.

In Table I are presented the measurements which were obtained in the study of the body of the trophozoite, in Table II the corresponding measurements of the nuclei, in Table III the area measurements of the nuclei, in Table IV the measurements of the cysts.

A study of the measurements of the body length in Table I shows an extreme composite range of 11 μ, viz., from 9.25 to 20.25 μ. It is noteworthy that the minimal ranges of the five forms are small, viz., only 2 μ, while the maximal range is much larger, viz., 5 μ. The range of the means, on the other hand, is also quite small, viz., 2.79 μ, the mean values for the five groups being nearer the minimal than the maximal figure, viz., 13.70 μ.

Earlier observers have given the following ranges for body length, but make no mention of the number of specimens that were measured; no determination of the mean has been previously recorded: Lambl 18–21 μ; Noc 16 μ; Perroncito 17 μ; Moritz and Hölzl 15.5–16.5 μ; Roos 15.5–17.5 μ; Salomon 10.0–18.0 μ; Piccardi 12 μ; Rodenwaldt 10.0–25.0 μ. It may be added that none of these observers mention the method of measuring which they employed, while our own measurements were all made from camera lucida drawings.

A survey of measurements of body breadth shows fluctuations within a narrower range than body length. This is what we would expect in as much as the length in *Giardia enterica* is so much greater than the breadth. The extreme composite range is here 5.25 μ, viz., a range between 5 and 10.25 μ. The mean values range between 6.31 and 7.87 μ and like the mean length favor the lower side, giving a composite mean of only 6.6 μ.

The figures given by earlier observers for body breadth follow: Lambl 8.6–11 μ; Noc 10.0 μ; Perroncito 10.0–12.0 μ; Moritz and Hölzl 10.0–12.5 μ; Roos 9.0–11.0 μ; Salomon 7.5–15.0 μ. These European forms, so far as length and breadth are concerned, thus seem to be a little larger than our American forms, though this conclusion is not

TABLE I.

Measurements which were obtained in the study of the body of the trophozoite from five different cases of Giardia enterica, together with the corresponding composite values.

	No. of Specimens.	Length of body.†			Breadth of body.†			Ratio of body length to breadth (B.L./B.B.).			Area of body.*		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	65	10.25	20.25	14.11	6.25	10.25	7.87	1.4	2.2	1.8	105	235	158
Maryland II	82	11.25	17.25	14.34	6.25	9.25	7.83	1.5	2.3	1.8	105	245	172
Maryland III	50	9.25	15.25	11.61	5.0	8.0	6.31	1.5	2.0	1.79	85	235	116
South Carolina	75	10.25	15.25	12.70	5.75	8.75	6.94	1.4	2.2	1.8	95	195	146
French	70	11.25	17.75	14.40	6.75	10.25	7.82	1.4	2.3	1.6	125	275	194
Composite	342	9.25	20.25	13.70‡	5.0	10.25	6.6‡	1.4	2.3	1.84‡	85	275	156‡

* These figures represent the area in square millimeters of camera lucida drawings corresponding to a magnification of 1600 diameters.

† In micra.

‡ The composite means were obtained, not from the means of the five cases, but from the measurements of the total number.

TABLE II.

Measurements in micra which were obtained in the study of the nuclei of the trophozoite of Giardia enterica from five different individuals, together with the corresponding composite values.

	No. of Specimens.	Length.			Breadth.			Ratio of length to breadth (NL/NB).			Nuclear area.†		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	1.6	2.6	2.14	1.0	2.0	1.41	1.0	2.0	1.51	7.5	32.5	17.0
Maryland II	50	1.6	2.6	2.04	1.0	1.8	1.44	1.0	1.8	0.91	7.5	22.5	14.4
Maryland III	50	1.5	3.0	2.10	1.0	2.0	1.58	1.0	2.0	1.46	7.5	32.5	16.0
South Carolina	50	1.5	2.6	2.16	1.0	2.0	1.47	1.0	2.1	1.49	7.5	27.5	16.0
French	50	1.5	3.0	2.18	1.0	2.0	1.50	1.0	2.1	1.45	7.5	27.5	16.8
Composite	250	1.5	3.0	2.14*	1.0	2.0	1.49*	1.0	2.1	1.36*	7.5	32.5	16.0*

† The area measurements are in terms of square millimeters and have reference to camera lucida drawings corresponding to a magnification of 1600 diameters.

* The composite means were obtained not from the means of the five cases, but from the measurements of the total number.

TABLE III.

Measurements of the area in square millimeters of camera lucida drawings of the body of Giardia enterica, of the two nuclei, and the ratio between body area and nuclear area (BA/NA). (Magnification 1600 times.)

The specimens were obtained from five different individuals. The composite values are also given.

	No. of Specimens.	Body area.			Nuclear area.			Ratio of nuclear to body area (B.A./N.A.).		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	105	235	158	7.5	32.5	17.0	4.5	20.0	10.4
Maryland II	50	105	245	172	7.5	22.5	14.4	6.5	20.0	12.2
Maryland III	50	85	235	116	7.5	32.5	16.0	5.5	13.0	8.6
South Carolina	50	95	195	146	7.5	27.5	16.0	5.0	16.0	9.7
French	50	125	275	194	7.5	27.5	16.8	6.0	25.0	12.7
Composite	250	85	275	156*	7.5	32.5	16.0*	4.5	25.0	10.7*

* The composite means were obtained not from the means of the five cases, but from measurements of the total number.

TABLE IV.

*Measurements in micra of the cysts of Giardia enterica, from five different individuals, together with the corresponding composite values.**

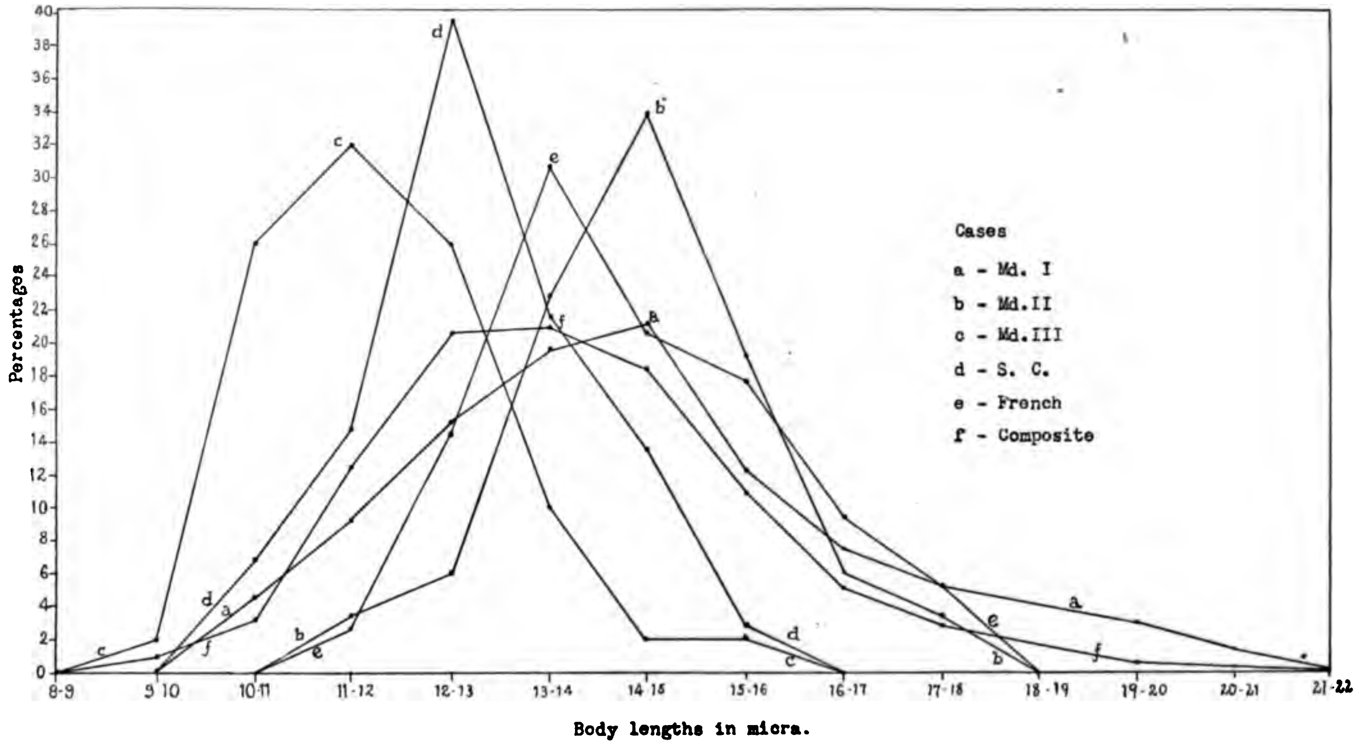
	No. of Specimens.	Length.			Breadth.			Ratio of length to breadth (L./B.).		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	8.0	13.0	10.55	6.0	8.5	7.47	1.0	1.8	1.41
Maryland III	50	9.0	12.5	11.0	6.5	10.0	8.24	1.1	1.8	1.33
Maryland IV	50	8.0	13.0	10.6	6.0	8.5	7.6	1.0	2.0	1.40
District of Columbia	50	9.0	12.5	10.8	6.0	10.0	8.28	1.0	1.6	1.33
Brazil	50	9.0	14.0	10.8	6.0	9.5	7.76	1.0	1.8	1.41
Composite	250	8.0	14.0	10.7†	6.0	10.0	7.47†	1.0	2.0	1.37†

* The measurements have reference to the cyst wall.

† The composite means were obtained not from the means of the five cases, but from measurements of the total number.

process takes place in some of the cysts (copulation cysts). v. Prowazek thus states "Occasionally it was noted that two individuals had come into apposition and had secreted a cyst wall." The four nuclei divided once more and the resultant products emerged from the peristomal border which was visible within the cyst in the form of two strongly curved lines. Subsequent changes could not be followed with certainty. Bensen's account of cyst formation (1908) was evidently influenced by that of v. Prowazek and Schaudinn. He states the following: "Cyst formation proceeds in such manner that two individuals come into ventral apposition and then encyst. Within the cyst they loose their flagella and a union of the nuclei results. Further changes have, however, not been observed." He regarded these copulation cysts with two nuclei as characteristic of the human form. Noc (1909) states that he was unable to observe either copulation or autogamy in the cysts. He points out that nuclear division within the cyst is not necessarily limited to the binary type, but may terminate in multiple division with a corresponding division of the cytoplasm. His subsequent account of what occurs is, however, somewhat confused and his illustrations are unfortunately so crude that they do not aid in the elucidation of his meaning. The "corps énigmatique" according to Noc undergoes longitudinal cleavage within the cyst and "one asks oneself whether the resulting chromatin filaments may not serve to regenerate the posterior flagella of the daughter giardias within the cyst."

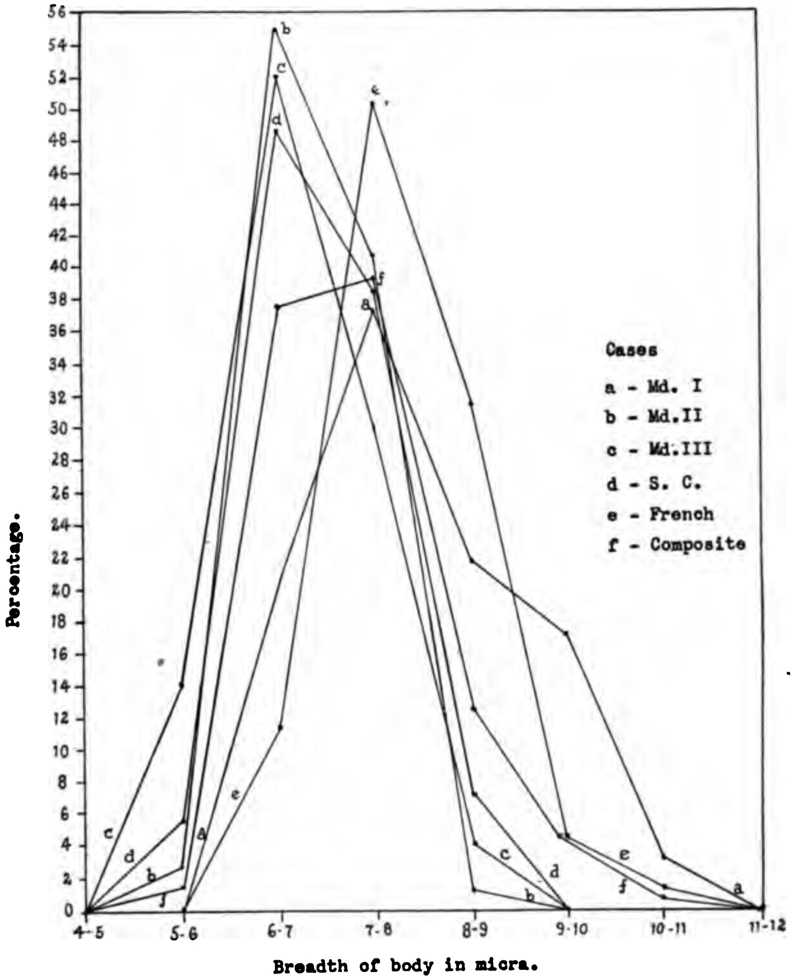
Rodenwaldt (1912), as I have stated, has given the first detailed account of the structure of the human cysts. Concerning the existence of copulation and autogamy cysts, as described by earlier writers, he thinks that such an assumption "seems somewhat audacious in view of the imperfect illustrations which have been furnished." He himself is willing to admit only "that he has evidence of division into two." "Some of the earlier stages of division," as he has seen them, "resemble the pictures of autogamy cysts of various observers and some of the more advanced stages the so-called copulation cysts." An outline of Rodenwaldt's own findings follows: Cyst formation is preceded by the appearance of a dense layer of fine granules beneath the arched dorsal surface; at the same time this latter increases in size. The dorsal portion of the animal extends ventrally over the sides, with the result that the contents of the peristome become folded in more and more. Rodenwaldt assumes that the cyst wall is formed from the granules just mentioned, because they disappear completely



CURVE 1. Showing the frequency values in percentage terms, of the body length of 342 specimens of *Giardia enterica* obtained from five different individuals, together with the corresponding composite values. The individuals are designated by the letters a, b, c, d and e, respectively, and the composite curve by the letter (f). The measurements are given in micra.

oos 9.0–12.0 μ length, 7.5–10.0 μ breadth; 5.0–7.5 μ breadth. These lues agree more closely with our own, but as has been said before e earlier observers have not recorded the number of cases studied, r the number of specimens that were measured, nor the method of easurement that was used.

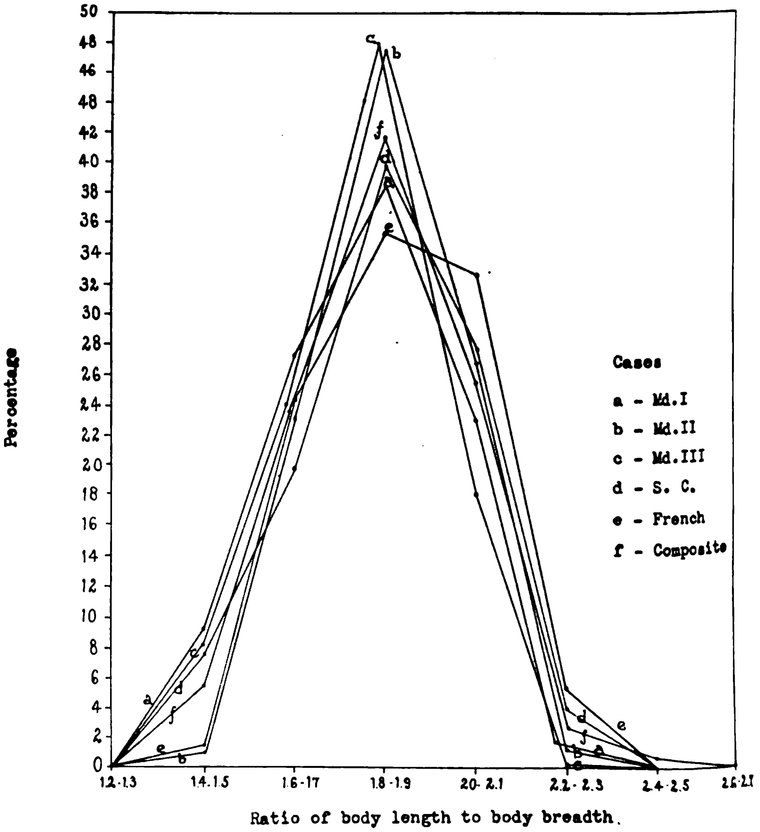
To turn now to a consideration of the various curves the writer ould state that they have been introduced in this paper largely as a



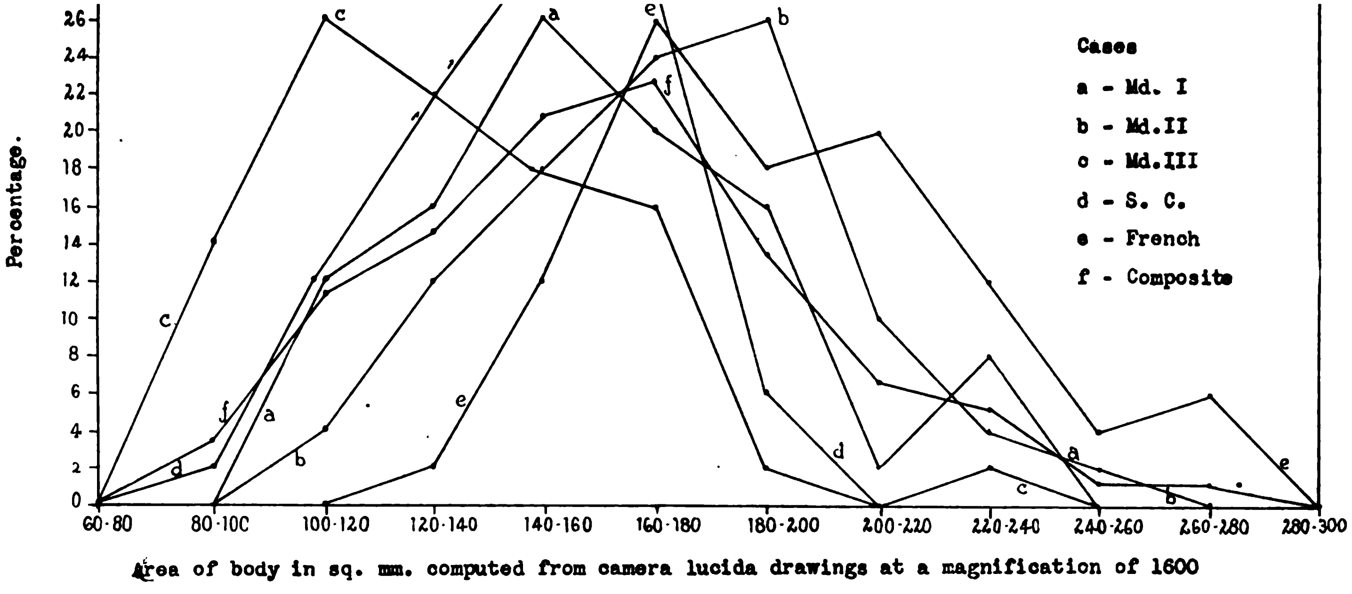
CURVE 2. Showing the frequency values in percentage terms of the body adth of the same specimens of *Giardia enterica* whose length has been re- ded in Curve 1, together with the corresponding composite values (f). The asurements are given in micra.

matter of record, and to serve as basis for a subsequent comparative study of the human and the rodent types of Giardia. It will accordingly suffice to emphasize at this place only the most striking features.

A glance at Curve 1 will show that the modes expressing the length of the trophozoites from the individual cases differ but little from the composite mode, the greatest difference being noted in Maryland III where it amounts to 2 μ, while in the remaining four cases it is only about 1 μ or less. On first view of the curves one might be tempted to speak of the existence of races of *Giardia enterica,* but while such may be shown to occur, the number of specimens that have been examined for the purpose of constructing the composite curve is



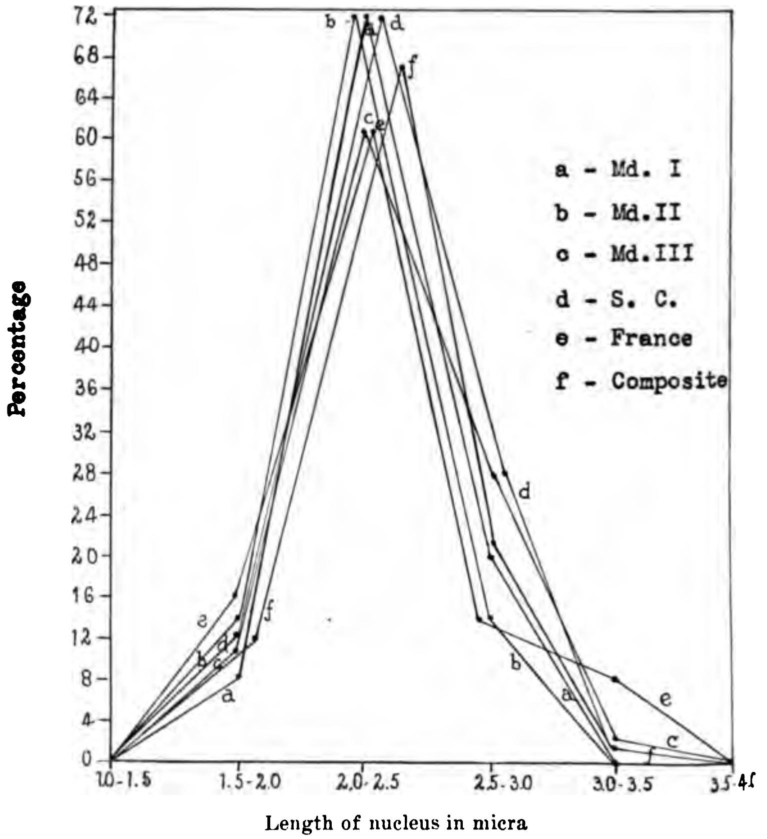
CURVE 3. Showing the frequency values in percentage terms of the relation between body length and body breadth (BL/BB) of the same specimens of *Giardia enterica,* whose length has been recorded in Curve 1, together with the corresponding composite values (f).



CURVE 4. Showing the frequency values in percentage terms of the body area of 250 specimens of *Giardia enterica*, obtained from the same individuals (a, b, c, d and e), together with the corresponding composite values (f). The measurements are given in terms of square millimeters and have reference to camera lucida drawings representing a magnification of 1600 diameters.

certainly not sufficient to warrant this conclusion. As in the case of the means the modes also lie nearer the lower than the higher values. It is clear, however, that there is a considerable range in size in the length values and that this factor alone cannot play an important rôle in a study of species. A small number of measurements would have but little meaning.

In Curve 2 are shown the frequency values in reference to breadth. It is clear that the differences occurring here are far less than those in the length of the organism, and we accordingly have much steeper and more uniform curves. The deviations of the individual modes



CURVE 5. Showing the frequency values in percentage terms of the nuclear length of 250 specimens of *Giardia enterica*, obtained from the same individuals (a, b, c, d and e), together with the composite values (f). The measurements are given in micra.

side, crossing over the posterior surface of the tail. Such specimens look as though the animal were covered with an oval shell, like a turtle.

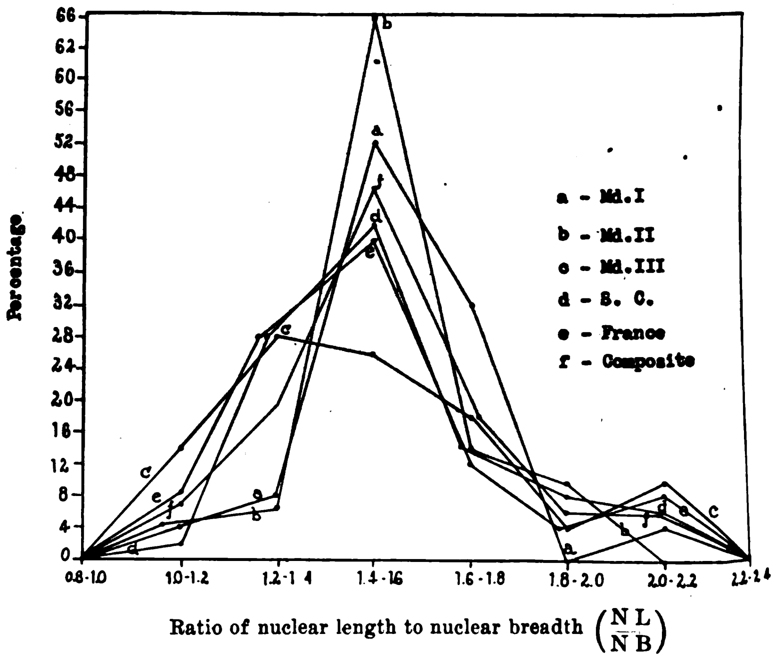
Rodenwaldt, who maintains the existence of two axostyles running to the caudal end of the body from a pair of basal granules lying in the depth of the cytostome between and a little anterior to the anterior poles of the nuclei, regards these as the "Hauptstützfibrillen," the principal supporting fibrils of the animal. The writer does not share this view. The writer, like Rodenwaldt, maintains the existence of two "axostyles," but in his estimation these are too delicate to serve as supporting structures, and he looks upon them purely as true intracytoplasmic continuations of the caudal flagella. In some specimens, particularly such as have not been sufficiently destained, the caudal halves of these structures are seen in close apposition and may then suggest a single body, but from the level of the parabasal body forward into the vestibule one can *always* see two fine lines which pursue a parallel course, the interval between which is always clear and distinct. In most thoroughly destained specimens there is no difficulty in distinguishing the course of these two lines posteriorly where they end not in one, but in two basal granules. (We shall revert to this question a little later on when discussing the neuromotor apparatus in detail.)

To recapitulate, the writer would look upon the periplast of the organism as an important supporting structure in itself. This is thickened along the inner and possibly the outer border of the lateral shields, and to some extent along the lateral border of the tail. Derived from it, no doubt, are the four separate fibrils which run along the border of the cytostome, viz., the two anterior and the two posterior peristomal fibrils. These are separate and apart from the neuromotor apparatus, which we shall now consider.

The neuro-motor apparatus.—The neuro-motor apparatus of the organism is composed of two nuclei, a number of centrally located basal granules connecting with the nuclei by means of rhizoplasts, four pairs of flagella, and peripherally located basal granules (see Text figure 1).

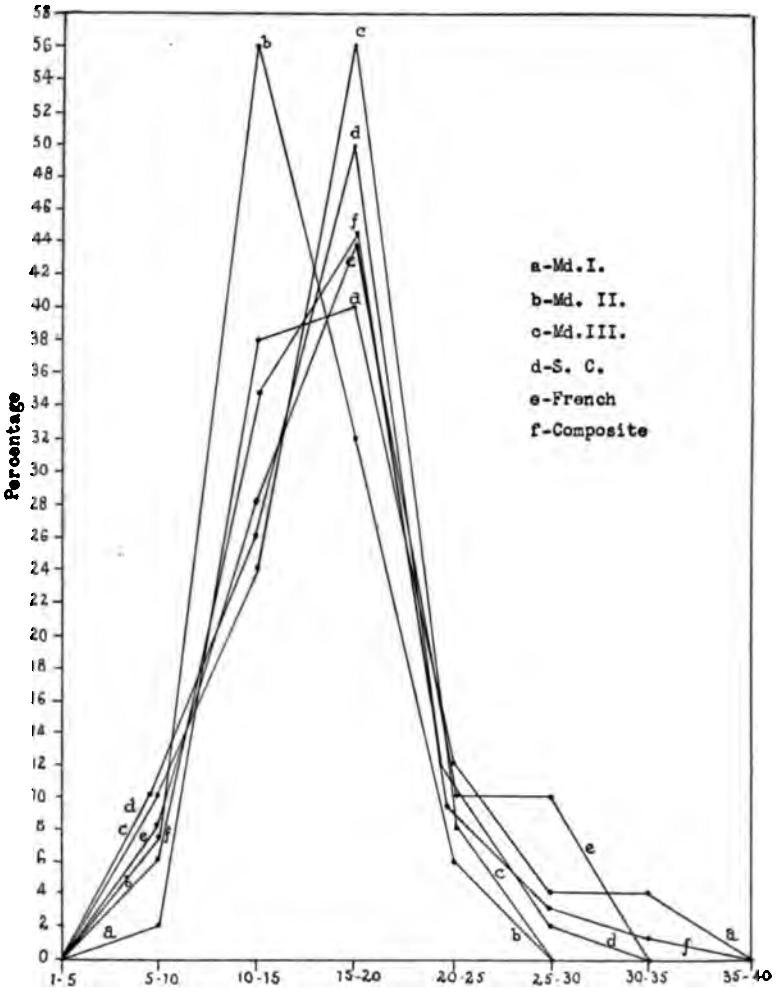
The two nuclei are seen in the depth of the cytostome. Their anterior poles converge slightly toward the longitudinal axis of the animal. Seen *en face,* as well as from the side, they are oval in form. The chromatin in the resting nucleus is gathered into a round or oval karyosome which is united to a fairly large somewhat crescentic appearing mass of chromatin, which is apparently attached to the well as the composite, correspond to the same ratio between the two factors.

What has been said in reference to the variations in the length of the organism applies also to the variations in the total body area, as is clear from a study of Curve 4. The differences in the values obtained are naturally more striking than the mere differences in length. Maryland III's mode for body area, like its mode for length, is strikingly small, and one is certainly tempted to look upon this as a race differing from the rest.



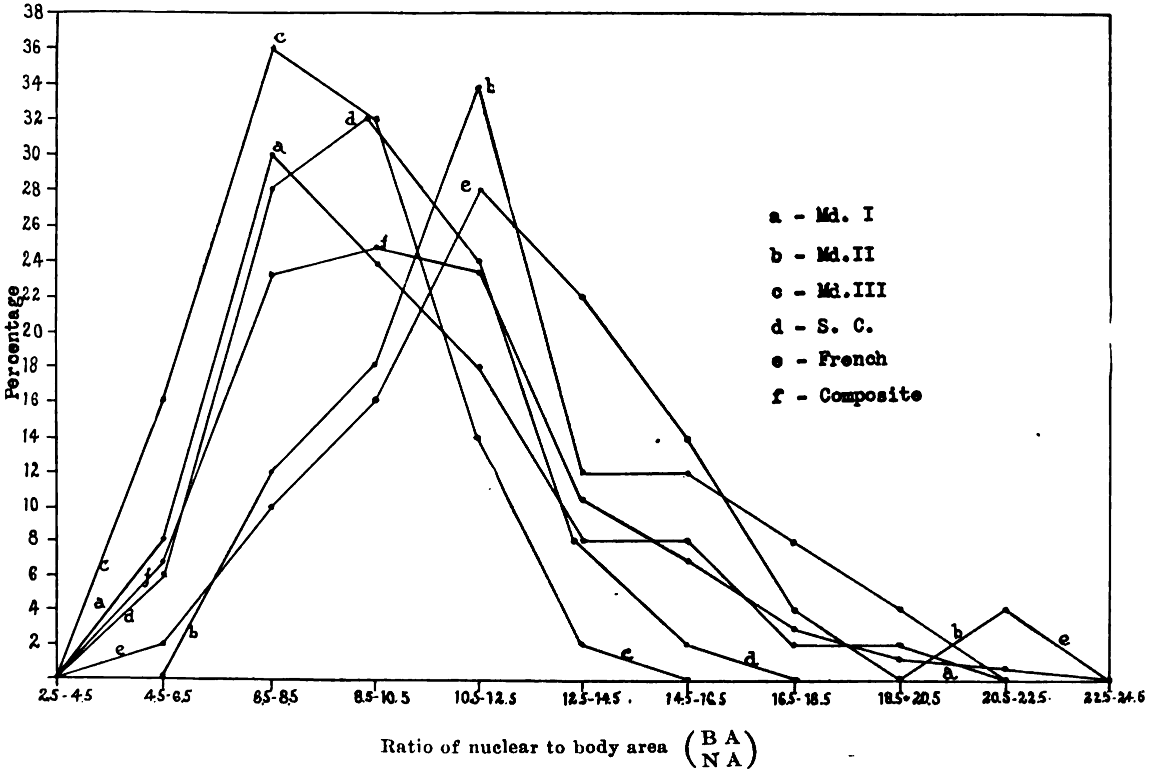
CURVE 7. Showing the frequency values in percentage terms of the relation between nuclear length and nuclear breadth (NL/NB) of the same specimens of *Giardia enterica*, whose length has been recorded in Curve 7, together with the corresponding composite values (f).

Curves 5, 6 and 7 have reference to the length, breadth and the ratio between the length and the breadth of the nucleus; as would be expected in such small structures, the values show very little variation. The modes expressing length and breadth practically correspond to the same measurements, viz., to a length of 2.0–2.5 μ and a breadth of 1.3–1.6 μ. The modes of the ratios are not as high as those of the



Combined area of nuclei in sq. mm.

Curve 8. Showing the frequency values in percentage terms of the area of two nuclei of the same specimens of *Giardia enterica*, whose body area has n recorded in Curve 4, together with the corresponding composite values. The asurements are given in terms of square millimeters, and have reference to the ne camera lucida drawings as in Curve 4.

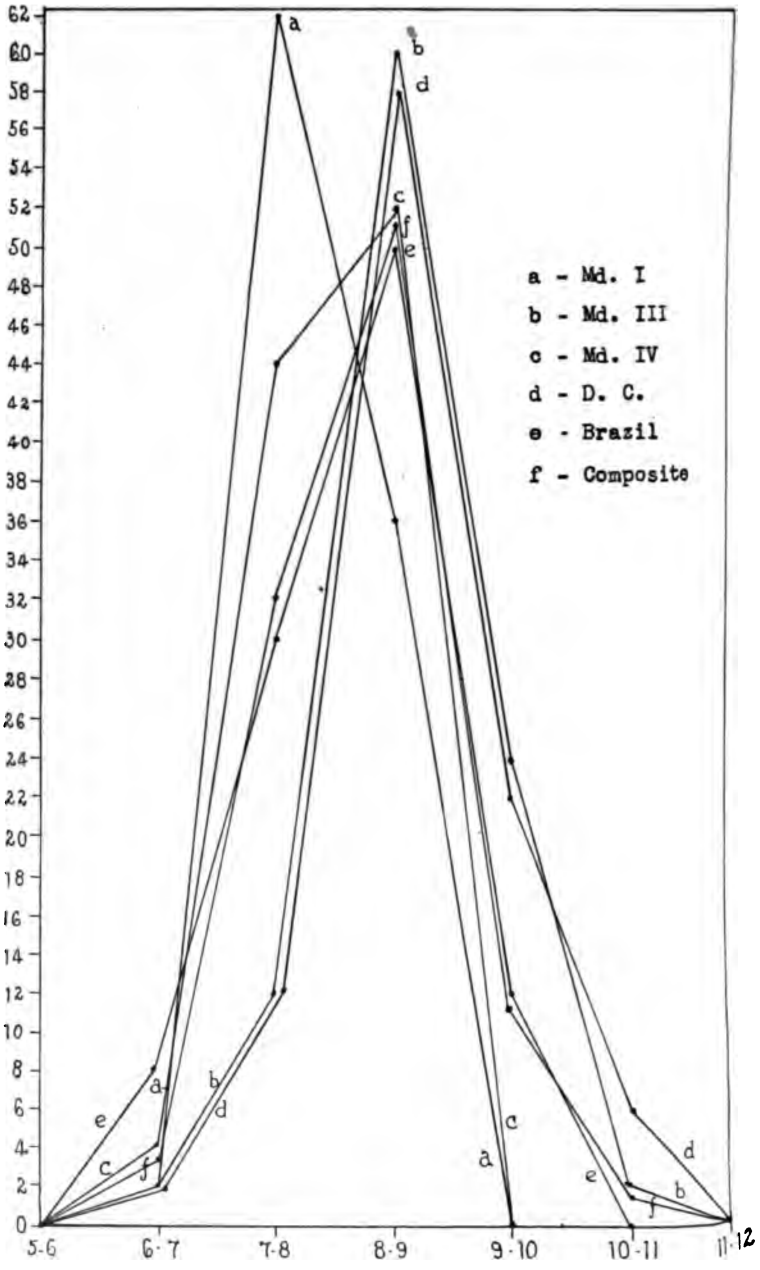


CURVE 9. Showing the frequency values in percentage terms of the relation between body area and nuclear area (BA/NA) of the same specimens, whose body area and nuclear area have been represented separately in Curves 4 and 8, together with the corresponding composite values (f).

Nuclear phase.	Parabasal body.	Appearance of axostyles.
1. Quiescent........	Full size	Distinctly double to caudal termination.
2. "	Almost gone	" " " " "
3. "	Absent	" " " " "
4. Eight granules in parallel rows...	Full size	" " " " "
5. Eight granules in parallel rows...	Absent	" " " " "
6. Late prophase....	"	" " " " "
7. Late prophase....	Full size	" " " " "
8. Late anaphase....	Full size	" " " " "
9. " "	Almost gone	" " " " "
10. Metaphase.......	Full size	" " " " "

Sometimes the body in question stains deeply, at other times scarcely at all. Sometimes its length equals or even exceeds that of the nucleus; at other times it is so small as to be scarcely discernible. The body is thus evidently not of a permanent nature, or perhaps it would be more appropriate to say the staining substance is not of a permanent nature. The fact, however, that when this staining substance is present it always seems to occupy the same position suggests the continued presence of a structure in which the staining substance is temporarily stored. Boeck (1919) has also come to this conclusion during his study of rodent forms. He states: "In about half of the flagellates the parabasal bodies appeared to be situated upon or in a definite area of the cytoplasm, limited by a membrane-like structure." This writer found that on staining with acid fuchsin and methyl green the bodies appeared red and the chromatin of the nuclei green, while in specimens stained with basic fuchsin the parabasals were not stained at all. With Wright's stain I found that the structures took the red in the same tone as did the nuclei and the flagella. With Mann's eosin-methyl blue mixture they were colored red, as were the karyosomes of the nuclei.

On staining Schaudinn preparations with Lugol's solution, Boeck found that a certain number showed a mahogany colored mass "filling all the parabasal-plasm," and he concludes that the function of the bodies is to secrete glycogen which is retained for subsequent use in the parabasal-plasm. While the writer agrees with Boeck that the contents of the parabasals *may* be composed of glycogen, he does not believe that the iodine test alone can be viewed as a criterion of the presence or absence of glycogen. It should be borne in mind that the so-called glycogen vacuoles of certain amoebae are not stained by iron-haematoxylin.

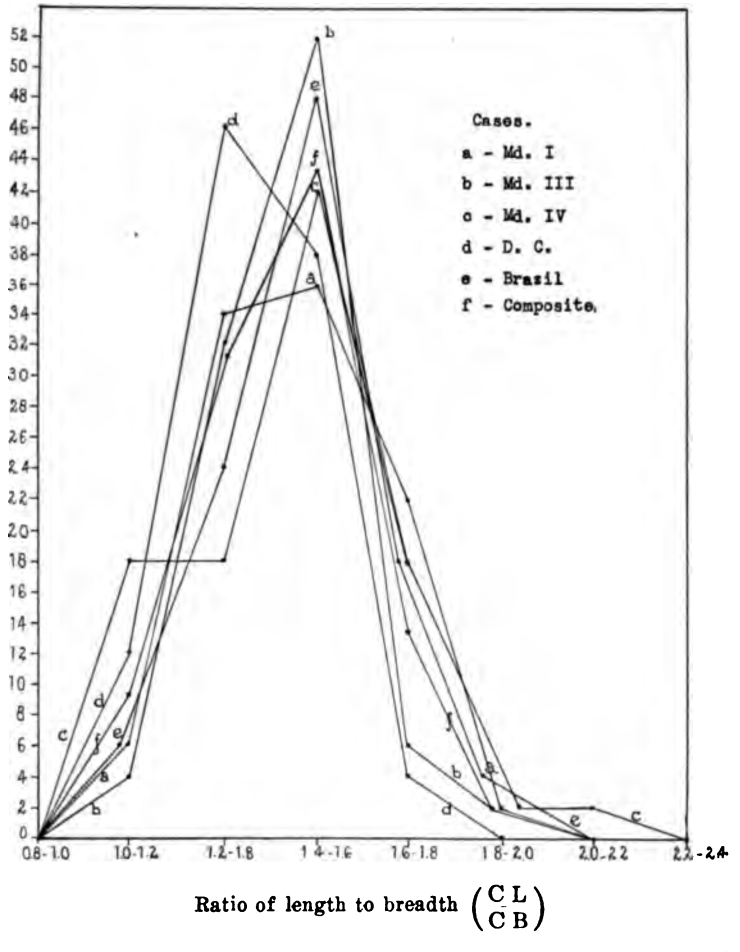


CURVE 11. Showing the frequency values in percentage terms of the breadth of the same encysted specimens of *Giardia enterica*, whose length has been recorded in Curve 10, together with the corresponding composite values (f). The measurements are given in micra and have reference to the cyst wall.

the composite mode is evidently the expression of the greater and variability in the length of the organism.

urves 10, 11 and 12 have reference to the variations in the frecy values of the length, breadth and the ratio between length and dth of the cysts.

urve 10 shows a smaller range in the length of the cysts and a r approach to the composite on the part of the individual modes do the corresponding values for the length of the trophozoite.



URVE 12. Showing the frequency values in percentage terms of the relation een cyst length and cyst breadth (CL/CB) of the same specimens of *Giardia ica*, whose length has been recorded in Curve 10, together with the cornding composite values (f).

The breadth values are shown in Curve 11 and are even more constant than those denoting the length of the cysts. The same is true of the ratios. The fluctuations of these latter are little more extensive than those in the case of the trophozoites. Of special interest is a comparison of the values found for Maryland I and Maryland III, which are represented in the two series. The modes for the ratios of these two forms is practically identical for the cysts and the trophozoites.

SUMMARY.

The trophozoite of Giardia enterica in ventral or dorsal view presents a pear-shaped appearance, the broad rounded portion corresponding to the anterior end and the attenuated fusiform portion to the posterior end. The ventral surface presents a cup like depression which occupies the greater portion of the anterior half of the body—the cytostome. The dorsal surface is arched.

The cytoplasm shows a delicate alveolar structure and is covered by a periplastic layer which appears denser along the dorsal surface and its triangular lateral extensions toward the ventral surface—the lateral shields. The ventral covering of the depressed diamond-shaped portion of the body between the lateral shields and extending to the tip of the tail, as well as the lining of the cytostome, appears to be more delicate. The cytostomal margin is stiffened by definite fibrils, of which there are four, viz., two anterior and two posterior peristomal fibrils. Between the anterior arched end of the organism and the anterior peristomal fibrils is a shallow groove. The anterior and posterior fibrils are not continuous with each other, leaving between them a lateral "joint." The inner margins of the lateral shields are bordered by periplastic ridges or fibrils which run forward, increasing in thickness, and reaching their greatest bulk within the vestibule, viz., the narrow passage which leads from the depressed diamond-shaped area into the cytostome.

The nuclei are two in number and located in the cytoplasm dorsal to the cytostome. They are elliptical in the resting stage and provided each with a karyosome, whence a fine linin fibril leads to a mass of chromatin which occupies a position along the anterior pole of the nucleus and possibly represents a centrosome. There is a definite nuclear membrane, but no peripheral chromatin. From each centrosome a rhizoplast curves slightly forward and inward to a granule, the blepharoplasts, of which there are two during the ordinary vegetative state of the animal. The two blepharoplasts are united with each other by a small fibrillar arch which carries a granule in the center—the interblepharoplastic granule.

The flagella are eight in number, arranged in pairs. The antero-laterals originate from the blepharoplasts. After first arching outward in their forward course they converge and cross in the "chiasm," where a granule can often be made out. They then run within the cytoplasm, apparently in the anterior groove and obscured by the anterior peristomal fibrils. They emerge from the body at the outer terminal ends of the latter, coming apparently from a basal granule. The postero-laterals likewise originate from the blepharoplasts, run along obscured by the periplastic fibrils of the "shields," and emerge where these terminate. No basal granules can be seen at these points. The caudal flagella pursue a straight though convergent course from the blepharoplasts to two basal granules at the tip of the sometimes bifurcated tail. Their intracystoplasmic portions are the "axostyles," of which there are accordingly two. The ventral flagella originate from two basal "rods" which are apparently attached to the "axostyles," one to each, at points just within the entrance to the "vestibule."

A little posterior to the point of origin of the ventral flagella and dorsal to the axostyles, within the cytoplasm, lies the "parabasal body." When fully developed this is club-shaped, and in stained preparations usually occupies an oblique position to the long axis of the body. In many specimens it is small, indistinct or apparently absent.

A biometric study of the trophozoites of *Giardia enterica* from five different individuals gave the following results:

In the *encysted state* the organism usually presents an oval, less commonly a rounded form. The cyst wall is dense, doubly contoured and highly refractive. Within the cyst wall the organism undergoes various changes associated with its division, and the appearance of the contents hence varies accordingly. In the vast majority of cysts four nuclei can be discerned; they are here round, not oval. They are usually grouped together, but in some specimens two of the nuclei may be seen "migrating" toward the posterior pole. In most specimens, even though nuclear division has taken place, only two blepharoplasts can be seen which are still united by the arched fibril carrying its central granule. The connection with the nuclei has apparently been lost. The cytoplasmic portions of the antero- and postero-lateral flagella are distinct and no longer obscured by periplastic fibrils or ridges. The " axostyles " also are seen without difficulty. Partial division of these structures is sometimes suggested in appearance, but can not be made out with certainty. The cytostome has disappeared and the periplastic fibrils (peristomal and border ridges of the shields) have lost their usual position and connection. Within the cytoplasm there appear markedly curved fibrils, each ending apparently in a granule and arranged in two sets of four each, which the writer is inclined to view as the free peristomal fibrils (evidently division of these must take place quite early following encystment). The slightly arched long and deeply staining fusiform rods which are seen in every specimen may possibly represent the freed border ridges of the shields.

The parabasal body recognizable as such is absent in cysts. Occasionally, though rarely, one meets with deeply staining coarsely granular masses scattered through the cystoplasm. Whether these are derivatives of the parabasal body is not known.

TABLE V.

Composite biometric values for the trophozoite of Giardia enterica.

Range of length.	Mean.	Mode.	Range of breadth.	Mean.	Mode.	Range of L./B.	Mean.	Mode.
A. Body:								
9.25–20.25	13.70	13.0–14.0	5.0–10.25	6.6	7.0–8.0	1.4–2.3	1.84	1.8–1.9
B. Nucleus:								
1.5–3.0	2.14	2.0–2.5	1.0–2.0	1.49	1.3–1.6	1.0–2.1	1.36	1.4–1.6

C. Area values:

Body Area:			Nuclear Area.			Body Area/Nuclear Area.		
Range.	Mean.	Mode.	Range.	Mean.	Mode.	Range.	Mean.	Mode.
85.0–275.0	156.0	160.0–180.0	7.5–32.5	16.0	15.0–20.0	4.5–25.0	10.7	8.5–10.5

A biometrical study of encysted forms of *Giardia enterica* gave the following results:

TABLE VI.

Composite values for the cysts of Giardia enterica.

Range of length.	Mean.	Mode.	Range of breadth.	Mean.	Mode.	Range of C.L./C.B.	Mean.	Mode.
8.0–14.0	10.7	11.0–12.0	6.0–10.0	7.47	8.0–9.0	1.0–2.0	1.37	1.4–1.6

In conclusion it is the writer's most agreeable duty to express his deep appreciation to Dr. Robert W. Hegner, the Director of the Department of Medical Zoology at the School of Hygiene, for his many acts of courtesy and his valuable aid and suggestions which were so freely offered in the course of the above study.

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EXPLANATION OF PLATES.

PLATE XXII.

FIG. 1. Reproduction of Lambl's first drawings, published in 1859.

FIG. 2. Lambl's more detailed illustrations of 1860. Note *g* which evidently represents a cyst.

FIGS. 3 and 4. Grassi's earliest illustrations of *Giardia enterica* (1883). Note the cyst pictured in Fig. 4*b* and the origin of the flagella in Fig. 4*a*.

FIG. 5. Bohne and v. Prowazek's illustrations of *Giardia enterica* (1908). Note their conception of the origin of the various flagella as pictured in *b*; *c* represents a cyst.

PLATE XXIII.

FIG. 1. Bensen's illustration of the type of Giardia occurring in man (1908).

FIG. 2. Two redrawn sketches from Noc; *a* represents the trophozoite, *b* a cyst (1909).

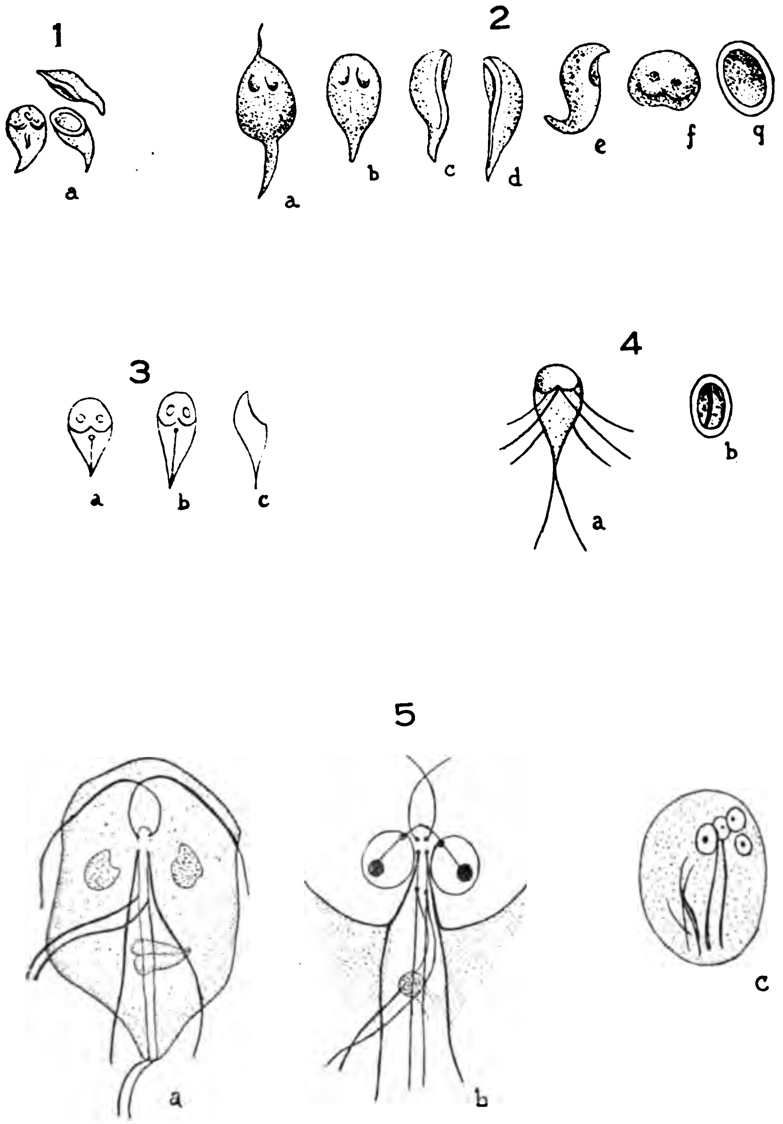

PLATE XXII.

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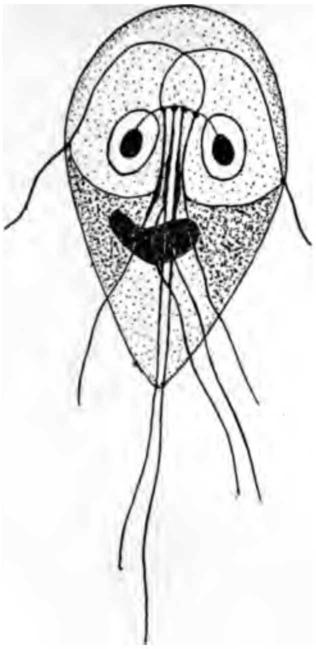

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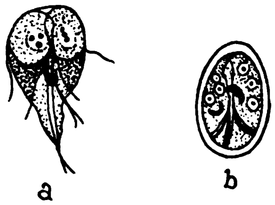

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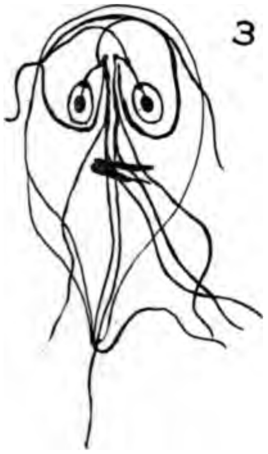

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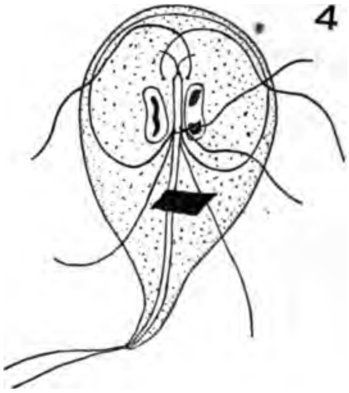


PLATE XXIII.

TABLE IV.

*Measurements in micra of the cysts of Giardia enterica, from five different individuals, together with the corresponding composite values.**

	No. of Specimens.	Length.			Breadth.			Ratio of length to breadth (L./B.).		
		Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.	Lowest.	Highest.	Mean.
Maryland I	50	8.0	13.0	10.55	6.0	8.5	7.47	1.0	1.8	1.41
Maryland III	50	9.0	12.5	11.0	6.5	10.0	8.24	1.1	1.8	1.33
Maryland IV	50	8.0	13.0	10.6	6.0	8.5	7.6	1.0	2.0	1.40
District of Columbia	50	9.0	12.5	10.8	6.0	10.0	8.28	1.0	1.6	1.33
Brazil	50	9.0	14.0	10.8	6.0	9.5	7.76	1.0	1.8	1.41
Composite	250	8.0	14.0	10.7†	6.0	10.0	7.47†	1.0	2.0	1.37†

* The measurements have reference to the cyst wall.

† The composite means were obtained not from the means of the five cases, but from measurements of the total number.

necessarily correct, in as much as the writers mentioned do not give any details regárding the number of specimens that were measured nor the method of measurement which was employed.

The values of the ratio between length and breadth, it will be noted, are remarkably constant, which would indicate that there is a definite correlation between the two. Figures for comparison do not exist.

A study of the body area values and a comparison with the corresponding figures for body length and body breadth shows that the fluctuations here are no doubt largely dependent upon the variations in the former. These measurements were made more particularly for the purpose of future comparative studies and need not be further considered at this place. It may be mentioned, however, that thus far no area measurements have been published in the study of any form of Giardia.

The nuclear measurements likewise need not be considered in detail at this place; they also are here recorded only for future purposes. It will be noted that in Table II the same forms of measurements are presented as were recorded in Table I in the study of the body of the organism. The area values in conformity to the length and breadth values are very constant, which no doubt is in part due to the minute size of these structures.

In Table III the body and nuclear area values have been reassembled and their ratio to each other determined. These figures show that the area of the body as a whole is from 8.6 to 12.7 times larger than the area occupied by the two nuclei together. These values also are largely intended for future comparative studies.

In Table IV measurements of *Giardia enterica* cysts are presented. These show a greater constancy than the corresponding measurements of the trophozoite. The length range between the lowest values of the five series is thus only 1 μ, and that between the highest values only 1.5 μ, giving a mean range of only 0.5 μ, as contrasted with 2 μ, 5 μ and 2.79 μ respectively, in the case of the trophozoite. This would suggest that for comparative purposes the study of the cysts may prove of greater importance than that of the trophozoites—at least in so far as the factor of length is concerned. The composite range for length was 8.0–14.0 μ (mean 6), for breadth 6.0–10.0 μ (mean 7.47), and for the ratio between length and breadth 1.0–2.0 μ (mean 1.37).

Earlier investigators have given the following values for the encysted form: Moritz and Hölzl 10.0–13.5 μ length, 10.0–10.5 μ breadth;

FIG. 3. Reproduction of one of Rodenwaldt's two indistinct drawings of the rophozoite (compare the text description) (1912).

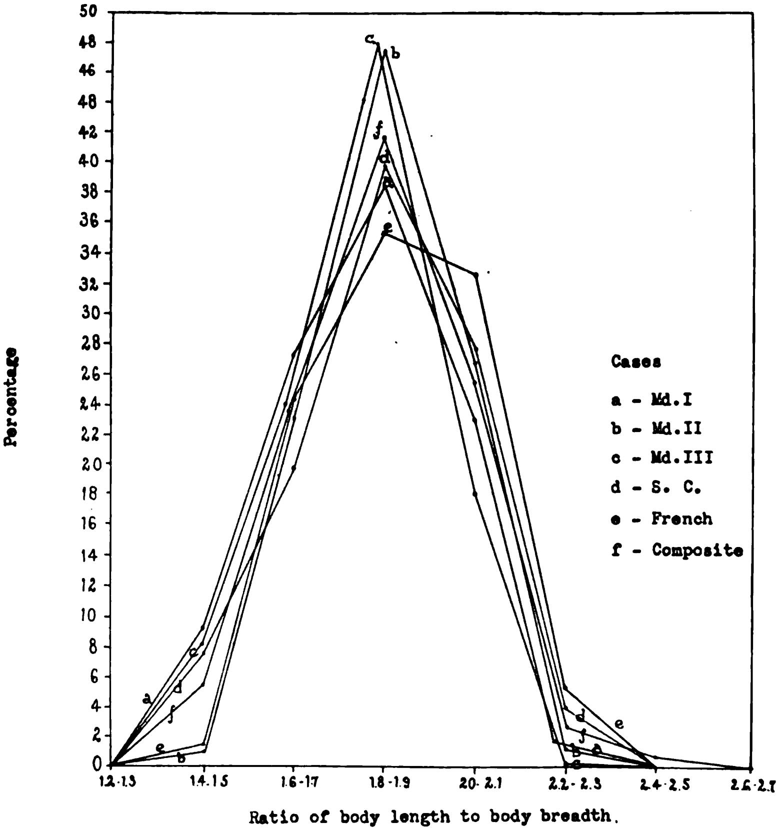
FIG. 4. One of a pair of dividing organisms from Wenyon and O'Connor, howing their conception of the structure of the organism (1917).

PLATE XXIV.

FIGS. 1–5. Reproductions of Rodenwaldt's drawings of cysts of *Giardia en-*
erica, showing the division and migration of the nuclei. Note the 'skeins' repre-
enting the peristomal fibrils and the 'guiding rods' supposedly of parabasal rigin (the latter pictured in 2 and 4) (1912).

matter of record, and to serve as basis for a subsequent comparative study of the human and the rodent types of Giardia. It will accordingly suffice to emphasize at this place only the most striking features.

A glance at Curve 1 will show that the modes expressing the length of the trophozoites from the individual cases differ but little from the composite mode, the greatest difference being noted in Maryland III where it amounts to 2 μ, while in the remaining four cases it is only about 1 μ or less. On first view of the curves one might be tempted to speak of the existence of races of *Giardia enterica*, but while such may be shown to occur, the number of specimens that have been examined for the purpose of constructing the composite curve is



CURVE 3. Showing the frequency values in percentage terms of the relation between body length and body breadth (BL/BB) of the same specimens of *Giardia enterica*, whose length has been recorded in Curve 1, together with the corresponding composite values (f).

degenerative changes as those which occurred in stained cysts. This was true for all the different species of cysts.

It was first noticed that the nuclei became distinct, that the cytoplasm appeared more coarsely granular and that slight plasmolysis took place. Later on plasmolysis increased, and in the *Endamoeba coli* cysts the 3 characteristic zones developed as described above (Fig. 1). The final stages of degeneration were similar to those which occurred in the stained cysts. These degenerative changes took place in cysts which had been kept in a favorable environment (in a bottle containing distilled water) for several months, and at all times they refused to take up the eosin stain.

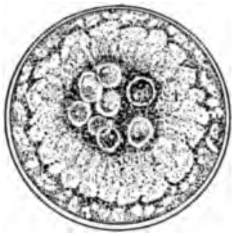


FIG. 1. *E. coli* cyst (dead). × 1860. Degeneration has resulted in the presence of the 3 zones in the cytoplasm.

But again, similar changes occurred within only a few days, when the cysts were in an unfavorable environment such as a stool not washed, or in cultures, in both of which there was an abundant bacterial growth resulting in the elaboration of putrefactive products of decomposition which hastened the degeneration of the cysts and led to the latter's early death.

From the work of Yoshida (1920) on the reproduction *in vitro* of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts, we note that many of the cysts which excysted had degenerated to a great extent and had plasmolized, for the cysts were described as possessing "a space, small or large . . . between the outer membrane and the cytoplasm," the dimensions of which varied greatly, "the largest observed being about half the size of the cysts." Certainly such changes were signs of degeneration similar to those observed in the cysts from the bottles, both those which stained with eosin, and those which remained refractive.

The question then arose, are all cysts alive which show marked degenerative changes and do not take up the eosin stain? This question is answered by making further reference to the experiments of

Yoshida. Just as 3 zones occurring in degenerated cysts of *Endamoeba coli* were noticed by the author, so were cysts possessing these changes found by Yoshida in his cultures (see Yoshida, 1920, pl. 29; Figs. 1, 11, 12). On one occasion, he observed the escape of the central core of protoplasm in one of these degenerated cysts upon the removal of the cyst wall. He questioned the viability of the freed protoplasmic mass, presumably one is led to believe, from the fact that it was motionless, as contrasted with the other motile excysted amoebae in his cultures.

We are led to conclude, therefore, that those cysts which show such marked degenerative changes, as very extensive plasmolysis and the possession of only a central core of cytoplasm, and in *E. coli* cysts 3 or less protoplasmic zones of degeneration, are dead (altho they do not stain with eosin, as Yoshida observed), for were such cysts alive then the one which Yoshida saw excyst, would have liberated a motile amoeba as many of his other cysts did which no doubt had not degenerated to the same extent.

This bit of experimental evidence furnishes the best answer to date, to the question whether or not all dead cysts stain with eosin-solution. And when we consider that Yoshida found that most of the amoeba obtained in his cultures were the result of excystation which was characterized by a "disintegration and dissolution of the cyst wall," we are also furnished with data to prove that cysts may undergo degeneration to the extent that their nuclei become visible, their cytoplasm more coarsely granular and less refractive, and that they may undergo some plasmolysis and still be viable and capable of excystation, but that those cysts are dead in which more extensive degenerative changes occur, such as alteration of the cytoplasm into zones as in *Endamoeba coli*, and plasmolysis to the extent that only a central core of protoplasm remains in other species. We can look upon slight changes as early degenerative phenomena, but upon marked changes as certain signs of death, even altho these changes occur in cysts which do not stain in the eosin-solution.

CRITERION OF VIABILITY.

All the data regarding the longevity of the cysts which will be presented in the following portion of this paper were collected with the understanding that those cysts were *alive* which did not stain in eosin-solution and showed but little or no plasmolysis and little or no loss in refractivity, while those cysts which stained in eosin-solution or remained unstained, but were markedly degenerated as described above were held to be *dead*. By supplementing the eosin-criterion of viability in this way with direct observation on the morphological changes which were present, it is believed that an almost certain differentiation was possible between dead and living cysts.

TECHNIQUE.

It has been shown by Kuenen and Swellengrebel (1913) and Boeck (1921) that the presence of bacteria and the chemical products of putrefaction exert a deleterious influence upon the vitality of the cysts; the different species of protozoan cysts were accordingly prepared so as to be comparatively free from these factors, by washing the stools containing the cysts.

The washing of the stool was accomplished as follows: The stool was immersed in distilled water and comminuted with a mechanical mixer. It was then strained through a couple of layers of bolting-cloth which removed the coarse fecal matter. The filtrate was allowed to stand for a few hours, during which time the cysts fell to the bottom of the container. The supernatant fluid containing bacteria and water-soluble putrefactive products was drawn off and more distilled water added. This procedure was repeated several times until the supernatant fluid was no longer colored or turbid, and then the cysts were transferred to a bottle with 50 to 100 c.c. of distilled water, and kept in a cool place where the temperature varied from 12° to 22° C. This low temperature inhibited excessive bacterial growth and retarded putrefaction to the point that it might be considered negligible.

The longevity studies were made upon the cysts which were kept under 2 different environments, (1) during the time they were kept in distilled water in their respective bottles and (2) while present in vaseline-slide preparations. In both environments the temperature was the same, 12° to 22° C. They were not exposed to sunlight nor kept in total darkness.

When examinations were made of the cysts from the bottle, sediment containing the cysts was withdrawn and a smear made in eosin-solution (1 : 1000 aqueous) to aid in differentiating the living from the dead cysts. Vaseline-slide preparations were made by taking a little sediment containing cysts from each of the bottles, making a smear in eosin-solution and sealing the specimens with vaseline to prevent evaporation. In the former case different cysts were observed at each examination since new preparations were made from the bottles for every observation, but in the latter case, the same groups of cysts were observed from time to time, as new vaseline-slide preparations were not made for each observation, but the original ones kept.

Cysts in bottles.

The results of the observations made upon the cysts in the bottles are given in Table I. Only the date of the last examination is given (4th column) on which viable cysts were encountered; how much longer these cysts remained viable was not determined. The number recorded in the 5th column represents the number of cysts which were viable out of the first 50 cysts seen in the examination. This number may be multiplied by 2 to obtain the per cent. of the cysts which were still living. In the 6th column is given the number of days which had elapsed since the cysts were passed with the feces i.e., their age.

TABLE I.

Longevity of the cysts in distilled water, in bottles.

Cysts.	Date passed.	Date stool washed.	Last examination.	No. cysts viable.	Age in days.
E. histolytica a......	Dec. 8, 1919	Dec. 11, 1919	Apr. 26, 1920	16	139
b......	" 14, 1920	" 15, 1920	May 16, 1921	1	153
E. coli.............	Jan. 15, 1920	Feb. 5, 1920	Sept. 16, 1920	6	244
Giardia intestinalis a.	Oct. 18, 1919	Oct. 22, 1919	Nov. 14, 1919	1	27
b.	Oct. 18, 1919	Oct. 22, 1919	Nov. 19, 1919	32	32
c.	Aug. 6, 1920	Aug. 6, 1920	Aug. 26, 1920	4	20
Chilomastix mesnili..	Jan. 27, 1920	Jan. 27, 1920	Aug. 2, 1920	44	187

From a comparison of the number of viable cysts, and the number of days elapsed since their passage, it is apparent that the cysts of *Chilomastix mesnili* might be expected to live the longest, for 88 per cent. of these were viable when they were 187 days old. This was no doubt due to the greater resistance of the *Chilomastix* cysts compared to the others, to the lower temperatures of 12° to 22° C., as was pointed out in the experiments to determine the thermal-death point of intestinal protozoan cysts (Boeck, 1921). In all probability the proportion of 44 viable cysts out of the first 50 cysts counted is higher than it should be because the empty cysts of *Chilomastix* are very difficult to see in a smear preparation on account of their small size and thin cyst wall, and they are therefore easily overlooked in an examination. In other words the death rate of these cysts was perhaps greater than the figures in Table I indicate. The cysts of *E. coli* were next in length of life, for 6 were alive 244 days after their passage from the body. The *E. histolytica* cysts were very resistant too, for 16 cysts had remained viable for 139 days. In the *b* series performed a year later, one viable cyst was seen 153 days after its passage in the stool on Dec. 14th, 1920. This cyst did not stain in eosin-solution; it had not undergone plasmolysis, and but a slight loss in refractivity. The nuclei were still invisible which is very unusual.

The *Giardia* cysts were not so long-lived. Three different series of observations were made upon cysts kept in bottles. One bottle of cysts from which the *b* series was made, was kept out of doors where the temperature was somewhat lower. To judge from the *a* and *c* series the *Giardia* cysts could not be expected to live much longer than 27 days, but in the *b* series (which was kept out of doors), they might have lived several days longer. This no doubt can be attributed to the influence of the cooler temperature out of doors which kept down the growth of the bacteria more effectively than the temperature indoors.

Cysts in vaseline-slide preparations.

Vaseline-slide preparations were kept at the same temperature of 12° to 22° C., at which the cysts in the bottles were kept. These preparations made it possible, as has been said above, to observe the same group of cysts from one examination to another, and furthermore to exclude all outside factors of influence from entrance. In these preparations the bacteria were greatly inhibited in their multiplication for the medium never became turbid, in fact it remained remarkably clear at all times.

Again, as in the above observations, the number of viable cysts out of the first 50 cysts encountered is recorded in the 5th column of Table II. The 6th column gives the age of these cysts dating from the passage of the stool. How much longer these cysts remained viable was not determined. They all showed a loss of refractivity and visible nuclei, but nevertheless they did not take up the eosin stain and the disintegrative changes were not such as to indicate that they were dead on the day of the last examination (Table I, column 4).

Fig. 3. Reproduction of one of Rodenwaldt's two indistinct drawings of the ophozoite (compare the text description) (1912).

Fig. 4. One of a pair of dividing organisms from Wenyon and O'Connor, howing their conception of the structure of the organism (1917).

Plate XXIV.

Figs. 1–5. Reproductions of Rodenwaldt's drawings of cysts of *Giardia en- rica*, showing the division and migration of the nuclei. Note the 'skeins' repre- nting the peristomal fibrils and the 'guiding rods' supposedly of parabasal rigin (the latter pictured in 2 and 4) (1912).

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ON THE LONGEVITY OF HUMAN INTESTINAL PROTOZOAN CYSTS.*

BY WILLIAM C. BOECK, PH.D.

(Received for publication, July 1, 1921.)

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INTRODUCTION.

The period of viability or longevity of the intestinal protozoan cysts of man is directly related to the period of infectivity of the cysts, and the prophylaxis of protozoan infections. After something is known of the length of life of these encysted organisms other experiments should be undertaken to determine their period of infectivity, i.e., the period during which, should the cysts be ingested, they are capable of producing an infection. But in the absence of knowledge of the period of infectivity, we may assume that while there is life, there is probable ability to produce infection, and hence the period of viability becomes of primary importance. It also follows that when the longevity of the cysts is known in full or in part, it will be possible to adopt effective prophylactic measures to combat the spread of protozoan infections.

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* From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

Before introducing the evidence regarding the longevity of the human intestinal cysts, it seems advisable to describe the changes through which the protozoan cysts pass when they are kept for a long time in a favorable environment, i.e., one which is conducive to long life, and when they are present but a short time in an unfavorable environment, in order that such changes may not be mistaken for signs of death in all cases, for some of these changes are only degenerative phenomena.

In connection with some experiments to determine the thermal-death point of the human intestinal protozoan cysts of *Endamoeba histolytica, Endamoeba coli, Giardia intestinalis* and *Chilomastix mesnili* (Boeck, 1921), it was decided to determine the fate of the cysts which had been killed by exposure to high temperature, and which stained with eosin (*dead*), and also the fate of the unstained cysts (*alive*) which were kept in bottles containing distilled water in a cool place, at a temperature of 12° to 22° C. As a result of these observations which were made over a period of 9 months, it was found that the stained cysts of *Endamoeba histolytica, Giardia intestinalis* and *Chilomastix mesnili* underwent extensive plasmolysis, the rate of which was very fast in *Giardia* cysts and slow in the cysts of *Chilomastix* and *Endamoeba histolytica.* As disintegration progressed only a small mass of protoplasm occupied the cysts, and this ultimately disappeared leaving the empty cyst. The cyst membrane too, disintegrated in time, leaving no trace of the cyst.

In the stained cysts of *Endamoeba coli,* a slight plasmolysis first took place, and this was followed by an alteration of the cytoplasm, to form 3 distinct zones (Fig. 1), viz., a central zone or core of protoplasm which usually contained all the nuclei, a narrow peripheral zone which appeared membranous and very coarsely granulated, and a middle zone which was almost without structure, as if fluid and separating the peripheral and central zones. Later on the peripheral zone disappeared as such and took on the appearance of the middle zone. The central core remained for a time, but later underwent dissolution too, leaving the cyst empty.

All these changes were held to be concomitant with degeneration, and their progression accomplished the complete disintegration of the cysts and their disappearance. These observations proved that all cysts which stain with eosin-solution are dead.

But the disintegrative changes just described were not peculiar to stained cysts alone, for as time went on it was found that many cysts which did not take up the eosin stain, underwent similar

degenerative changes as those which occurred in stained cysts. This was true for all the different species of cysts.

It was first noticed that the nuclei became distinct, that the cytoplasm appeared more coarsely granular and that slight plasmolysis took place. Later on plasmolysis increased, and in the *Endamoeba coli* cysts the 3 characteristic zones developed as described above (Fig. 1). The final stages of degeneration were similar to those which occurred in the stained cysts. These degenerative changes took place in cysts which had been kept in a favorable environment (in a bottle containing distilled water) for several months, and at all times they refused to take up the eosin stain.

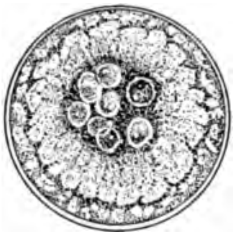

FIG. 1. *E. coli* cyst (dead). × 1860. Degeneration has resulted in the presence of the 3 zones in the cytoplasm.

But again, similar changes occurred within only a few days, when the cysts were in an unfavorable environment such as a stool not washed, or in cultures, in both of which there was an abundant bacterial growth resulting in the elaboration of putrefactive products of decomposition which hastened the degeneration of the cysts and led to the latter's early death.

From the work of Yoshida (1920) on the reproduction *in vitro* of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts, we note that many of the cysts which excysted had degenerated to a great extent and had plasmolized, for the cysts were described as possessing "a space, small or large . . . between the outer membrane and the cytoplasm," the dimensions of which varied greatly, "the largest observed being about half the size of the cysts." Certainly such changes were signs of degeneration similar to those observed in the cysts from the bottles, both those which stained with eosin, and those which remained refractive.

The question then arose, are all cysts alive which show marked degenerative changes and do not take up the eosin stain? This question is answered by making further reference to the experiments of

Yoshida. Just as 3 zones occurring in degenerated cysts of *Endamoeba coli* were noticed by the author, so were cysts possessing these changes found by Yoshida in his cultures (see Yoshida, 1920, pl. 29; Figs. 1, 11, 12). On one occasion, he observed the escape of the central core of protoplasm in one of these degenerated cysts upon the removal of the cyst wall. He questioned the viability of the freed protoplasmic mass, presumably one is led to believe, from the fact that it was motionless, as contrasted with the other motile excysted amoebae in his cultures.

We are led to conclude, therefore, that those cysts which show such marked degenerative changes, as very extensive plasmolysis and the possession of only a central core of cytoplasm, and in *E. coli* cysts 3 or less protoplasmic zones of degeneration, are dead (altho they do not stain with eosin, as Yoshida observed), for were such cysts alive then the one which Yoshida saw excyst, would have liberated a motile amoeba as many of his other cysts did which no doubt had not degenerated to the same extent.

This bit of experimental evidence furnishes the best answer to date, to the question whether or not all dead cysts stain with eosin-solution. And when we consider that Yoshida found that most of the amoeba obtained in his cultures were the result of excystation which was characterized by a "disintegration and dissolution of the cyst wall," we are also furnished with data to prove that cysts may undergo degeneration to the extent that their nuclei become visible. their cytoplasm more coarsely granular and less refractive, and that they may undergo some plasmolysis and still be viable and capable of excystation, but that those cysts are dead in which more extensive degenerative changes occur, such as alteration of the cytoplasm into zones as in *Endamoeba coli,* and plasmolysis to the extent that only a central core of protoplasm remains in other species. We can look upon slight changes as early degenerative phenomena, but upon marked changes as certain signs of death, even altho these changes occur in cysts which do not stain in the eosin-solution.

CRITERION OF VIABILITY.

All the data regarding the longevity of the cysts which will be presented in the following portion of this paper were collected with the understanding that those cysts were *alive* which did not stain in eosin-solution and showed but little or no plasmolysis and little or no loss in refractivity, while those cysts which stained in eosin-solution or remained unstained, but were markedly degenerated as described above were held to be *dead*. By supplementing the eosin-criterion of viability in this way with direct observation on the morphological changes which were present, it is believed that an almost certain differentiation was possible between dead and living cysts.

TECHNIQUE.

It has been shown by Kuenen and Swellengrebel (1913) and Boeck (1921) that the presence of bacteria and the chemical products of putrefaction exert a deleterious influence upon the vitality of the cysts; the different species of protozoan cysts were accordingly prepared so as to be comparatively free from these factors, by washing the stools containing the cysts.

The washing of the stool was accomplished as follows: The stool was immersed in distilled water and comminuted with a mechanical mixer. It was then strained through a couple of layers of bolting-cloth which removed the coarse fecal matter. The filtrate was allowed to stand for a few hours, during which time the cysts fell to the bottom of the container. The supernatant fluid containing bacteria and water-soluble putrefactive products was drawn off and more distilled water added. This procedure was repeated several times until the supernatant fluid was no longer colored or turbid, and then the cysts were transferred to a bottle with 50 to 100 c.c. of distilled water, and kept in a cool place where the temperature varied from 12° to 22° C. This low temperature inhibited excessive bacterial growth and retarded putrefaction to the point that it might be considered negligible.

The longevity studies were made upon the cysts which were kept under 2 different environments, (1) during the time they were kept in distilled water in their respective bottles and (2) while present in vaseline-slide preparations. In both environments the temperature was the same, 12° to 22° C. They were not exposed to sunlight nor kept in total darkness.

When examinations were made of the cysts from the bottle, sediment containing the cysts was withdrawn and a smear made in eosin-solution (1 : 1000 aqueous) to aid in differentiating the living from the dead cysts. Vaseline-slide preparations were made by taking a little sediment containing cysts from each of the bottles, making a smear in eosin-solution and sealing the specimens with vaseline to prevent evaporation. In the former case different cysts were observed at each examination since new preparations were made from the bottles for every observation, but in the latter case, the same groups of cysts were observed from time to time, as new vaseline-slide preparations were not made for each observation, but the original ones kept.

Cysts in bottles.

The results of the observations made upon the cysts in the bottles are given in Table I. Only the date of the last examination is given (4th column) on which viable cysts were encountered; how much longer these cysts remained viable was not determined. The number recorded in the 5th column represents the number of cysts which were viable out of the first 50 cysts seen in the examination. This number may be multiplied by 2 to obtain the per cent. of the cysts which were still living. In the 6th column is given the number of days which had elapsed since the cysts were passed with the feces i.e., their age.

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c.	Aug. 6, 1920	Aug. 6, 1920	Aug. 26, 1920	4	20
Chilomastix mesnili..	Jan. 27, 1920	Jan. 27, 1920	Aug. 2, 1920	44	187

From a comparison of the number of viable cysts, and the number of days elapsed since their passage, it is apparent that the cysts of *Chilomastix mesnili* might be expected to live the longest, for 88 per cent. of these were viable when they were 187 days old. This was no doubt due to the greater resistance of the *Chilomastix* cysts compared to the others, to the lower temperatures of 12° to 22° C., as was pointed out in the experiments to determine the thermal-death point of intestinal protozoan cysts (Boeck, 1921). In all probability the proportion of 44 viable cysts out of the first 50 cysts counted is higher than it should be because the empty cysts of *Chilomastix* are very difficult to see in a smear preparation on account of their small size and thin cyst wall, and they are therefore easily overlooked in an examination. In other words the death rate of these cysts was perhaps greater than the figures in Table I indicate. The cysts of *E. coli* were next in length of life, for 6 were alive 244 days after their passage from the body. The *E. histolytica* cysts were very resistant too, for 16 cysts had remained viable for 139 days. In the *b* series performed a year later, one viable cyst was seen 153 days after its passage in the stool on Dec. 14th, 1920. This cyst did not stain in eosin-solution; it had not undergone plasmolysis, and but a slight loss in refractivity. The nuclei were still invisible which is very unusual.

The *Giardia* cysts were not so long-lived. Three different series of observations were made upon cysts kept in bottles. One bottle of cysts from which the *b* series was made, was kept out of doors where the temperature was somewhat lower. To judge from the *a* and *c* series the *Giardia* cysts could not be expected to live much longer than 27 days, but in the *b* series (which was kept out of doors), they might have lived several days longer. This no doubt can be attributed to the influence of the cooler temperature out of doors which kept down the growth of the bacteria more effectively than the temperature indoors.

Cysts in vaseline-slide preparations.

Vaseline-slide preparations were kept at the same temperature of 12° to 22° C., at which the cysts in the bottles were kept. These preparations made it possible, as has been said above, to observe the same group of cysts from one examination to another, and furthermore to exclude all outside factors of influence from entrance. In these preparations the bacteria were greatly inhibited in their multiplication for the medium never became turbid, in fact it remained remarkably clear at all times.

Again, as in the above observations, the number of viable cysts out of the first 50 cysts encountered is recorded in the 5th column of Table II. The 6th column gives the age of these cysts dating from the passage of the stool. How much longer these cysts remained viable was not determined. They all showed a loss of refractivity and visible nuclei, but nevertheless they did not take up the eosin stain and the disintegrative changes were not such as to indicate that they were dead on the day of the last examination (Table II, column 4).

TABLE II.

Longevity of the cysts in the vaseline-slide preparations.

Cysts.	Date passed.	Date of vaseline-slide.	Date examined.	No. of cysts viable.	Age in days.
E. histolytica	Dec. 8, 1919	Feb. 5, 1920	July 21, 1920	12	211
E. coli	Jan. 15, 1920	Feb. 12, 1920	May 17, 1920	9	124
Giardia intestinalis a.	Oct. 18, 1919	Oct. 24, 1919	Dec. 23, 1919	10	66
b.	Aug. 6, 1920	Aug. 6, 1920	Sept. 7, 1920	2	22
Chilomastix mesnili	Jan. 27, 1920	Feb. 6, 1920	Sept. 16, 1920	45	232

From a study of Table II, it will be seen that the *Chilomastix* cysts again proved themselves able to live longer than the other species of cysts. And again, it may be said, that the proportion of viable cysts out of the first 50 cysts observed is no doubt higher than it should be for the same reason as given above, namely that empty cysts are easily overlooked. Nevertheless it was apparent that these cysts were out-living the others, and this (possibly?) by reason of their strong resistance to the lower temperatures.

It was a surprise, that the cysts of *E. histolytica* lived longer in the vaseline-slide preparations than those of *E. coli*. I believe, however, this does not actually occur in life, but that the opposite is true instead, as was found to be the case with the cysts in the bottles. It must have been that the *E. coli* cysts in the vaseline-slide preparations were affected by some detrimental influences either before the preparations were made, or afterward, that resulted in their early death.

Again, the *Giardia* cysts were shown to die at an early age compared to the others, but it is significant that 10 cysts were still viable at the end of 66 days (series *a*), and this was considerably longer than the cysts remained viable in the bottles. In another series, *b* (Table II), the cysts lived but little longer than 32 days, in all probability, and this, in view of the data in the *a* series can only be attributed to the influence of some detrimental factor. It is known for instance, that urine has a decidedly deleterious effect upon these cysts especially, and it is conceivable that it was previous contact with this factor while in the stool or some other influence, which resulted in the early death of these cysts in the *b* series.

Survival of cysts in fly's intestine.

Root (1921) has shown that cysts of the human intestinal protozoa may remain apparently viable for many hours in the intestine of the fly. It seems significant to point out that those cysts which survived the longest in the fly's intestine were of the same species as those which were found to live the longest in the experiments and observations given above. The period of survival of the cysts in the fly's intestine was proportional to their longevity outside when immersed in distilled water, altho the length of life of the cysts in the intestine was ever so much shorter. For the sake of convenience in comparing the results which Root obtained with those on longevity the following table is given. The number of viable cysts out of the first 50 observed is recorded at the time of the last examination.

TABLE III.

Period of viability of cysts in fly's intestine, as compared with their survival in distilled water in bottles and in vaseline-slide preparations.

	In fly's intestine.		In bottles or vaseline preparations.	
	No. cysts viable.	End of	No. cysts viable.	End of
Giardia intestinalis	1—(1 series)	16 hours	10	66 days
E. histolytica	1—(1 series)	49 "	12	211 "
E. coli	1—(1 series)	52 "	6	244 "
Chilomastix mesnili	2—(1 series)	80 "	45	232 "

It is to be seen at once that the cysts of *Giardia intestinalis* live the shortest of all the cysts, whether in the fly's intestine or outside when immersed in distilled water. Then follow the cysts of *E. histolytica, E. coli* and lastly *Chilomastix mesnili.*

Assuming that the factors in the intestine of the flies used in the experiments by Root equally influenced all the species of cysts after their ingestion, the period of survival becomes an expression of the comparative resistance of the different species of cysts. Of all the factors present in the intestine of the flies, fermentation caused by the bacteria in the intestine was probably chiefly responsible for the death of the cysts, as the temperature was low, in as much as this tends to be the same as that of the surrounding atmosphere, therefore room temperature (27° C.). It is altogether likely that the digestive juices may also have operated against the survival of the cysts. Therefore we may rightly assume that the cysts of *Chilomastix mesnili* were more resistant than the other species of cysts, in view of the sameness of the environment. The *Chilomastix* cysts survived longer than the other cysts in the fly's intestine, very probably on account of their greater resistance to the low temperature (27° C. or less), all other factors being equal, as was shown to be the case in the thermal-death point determinations (Boeck, 1921). For this same reason, in all probability, the *Chilomastix* cysts lived longer than the other species in the experiments given above to determine their longevity.

Intracystic development.

There was no evidence of definite intracystic development in the viable cysts which had been kept in the supply bottles and in the vaseline-slide preparations. In the observations made upon the endamoebic cysts, no evidence whatsoever occurred to indicate that single nucleated cysts developed into bi-nucleated cysts and bi-nucleated cysts into 4-nucleated cysts and so on, as was thought to occur by some of the earlier investigators.

I believe that the finding of 1 and 2-nucleated cysts of *E. histolytica* in the feces as well as of 1, 2, and 4-nucleated cysts of *E. coli* only signifies that these cysts were prevented from reaching the full development of 4-nucleated cysts in the case of the former parasite and of 8-nucleated cysts in the latter, by their premature ejection from the human body and that they develop no further unless their environment closely simulates that of the human intestine, a possible exception being those cysts in which the nuclei are in the midst of mitosis, when they have become transformed into spindles.

In view of the longevity observations described above it seems that the reproduction *in vitro*, as described by Yoshida (1920) for *E. coli* and *E. histolytica,* represents abnormal or degenerative phenomena brought about by the fermentative action of the bacteria in his artificial cultures, causing the newly formed amoebae to die on the first or second day following excystation "from the effects of putrefaction in the culture medium." It is only reasonable to assume that the bacteria were responsible for the disintegration of the cysts by progressive plasmolysis, some of which excysted to give rise to abnormal amoebae.

Although I was unable to detect any development in the cysts of

Giardia and *Chilomastix,* failure in the latter case must have been due to some unknown cause, since Kofoid and Swezy (1920) found that many *Chilomastix* cysts underwent development after they had been passed from the human body. The development resulted in the mitotic division of the nucleus and in the formation of another neuromotor apparatus. The only changes I saw, however, in the *Chilomastix* cysts which had been kept for 232 days was the presence of small, usually spheroidal-shaped chromatin-staining masses in the cytoplasm. These masses occupied no definite position in the cysts, but were situated here and there. Generally they were from 1 to 4 in number. The origin of these bodies was not indicated. They may represent chromatoidal bodies, the remains of the disintegrated neuromotor apparatus, or what is less probable chromatin material which had been extruded into the cytoplasm from the nucleus. The nucleus in these cysts possessed a large central karyosome, or at times, 1 to 3 smaller chromatin masses. A centrosome was demonstrable at the anterior pole of the nucleus on the nuclear membrane. The neuromotor apparatus had partly disappeared in many cysts, for not all the structures were visible which go to make up this fibrillar complex.

Period of infectivity.

With the knowledge at hand that the cysts of the human intestinal protozoa may remain viable for several months as has been shown with the use of the best criteria of viability available, it is of utmost importance to determine the period of infectivity as was mentioned in the opening paragraph of this paper. In all probability the period of infectivity does not coincide with the longevity of the cysts, but the proof is very meager at present to substantiate this statement. The most valuable evidence we have pertains to ingestion experiments upon man and kittens with cysts of *Endamoeba histolytica, Endamoeba coli* and *Giardia intestinalis.*

Darling (1913) after diluting the feces containing cysts of *E. histolytica* with 10 volumes of sterile tap water and allowing them to stand for 3 days, was unable to infect 2 kittens. But this might have been due as he states, "to an insufficiency of cysts."

Walker and Sellards in 1913, however, were able to infect men with *E. histolytica* with cysts which had been kept for 2 days at tropical temperature, and with *E. coli* with cysts which had been kept 10 days. The cysts were kept moist in both cases.

Other investigators have made observations on the longevity of the intestinal protozoan cysts of man, among them Penfold, Woodcock and Drew (1916), who kept the cysts of *E. histolytica* alive in running water for 15 days; Thomson and Thomson (1916), who observed viable cysts of *E. histolytica* in moist feces after 16 days, and Wenyon and O'Connor (1917) after a month, but none of these workers conducted much needed experiments to see whether the viable cysts were also infectious for man or laboratory animals.

The experiments of Walker (Sellards) in 1913 were conducted upon human beings and his results therefore are especially noteworthy. It seems probable, however, in view of the fact that cysts may be kept apparently viable for a long time that the period of infectivity for *E. histolytica* would be found longer than 2 days and for *E. coli* longer than 10 days had more experiments been conducted of a similar nature to those performed by this investigator.

The period of infectivity of *Giardia intestinalis* is considerably longer than that known thus far for the entamoebic cysts; it appears to coincide with the period of viability. Fantham and Porter (1916) were able to infect kittens with cysts of this parasite which had been kept for 74 days after they had been washed free from fecal debris. This period of infectivity is even greater than the longest period of viability, which was 66 days, obtained in the experiments detailed above, in the *a* series of Table II.

Until we are in possession of more definite facts regarding the period of infectivity of all intestinal protozoan cysts of man, and especially of *E. histolytica*, all prophylactic measures should look toward the destruction of the feces within 2 days if possible, since as we have seen, cysts may be still infectious within this period, if ingested.

Man contracts his infection through accidental contact with the feces containing the cysts and ingesting the latter or probably through the eating of food or drinking of water in which cysts have been deposited by flies which have visited privies and eaten feces containing cysts. These methods of dissemination emphasize the importance, as other investigators have shown, of adopting such preventative measures of sanitation as will exclude the flies from the feces, and also to dispose of the latter as soon as possible after defecation.

SUMMARY.

1. Accepting the eosin-solution, supplemented by morphological observations as a criterion of viability, the longevity was determined for 4 different species of human intestinal protozoan cysts, under 2 different environments.

2. Immersed in distilled water, contained in bottles and kept at a temperature of 12° to 22° C., cysts of *E. histolytica* were found viable at the end of 153 days; *E. coli* at the end of 244 days; *Giardia intestinalis* at the end of 32 days and *Chilomastix mesnili* at the end of 187 days.

3. In eosin-stained wet preparations, sealed with vaseline, cysts of *E. histolytica* were viable at the end of 211 days; *E. coli,* 124 days; *Giardia intestinalis,* 66 days and *Chilomastix mesnili* at the end of 232 days.

4. While the results of the observations did not reveal the actual length of life of each species of cyst, yet they demonstrated that the cysts remained viable for a considerable period of time and that under similar conditions of a low temperature and the exclusion of bacterial influences, the cysts of *Chilomastix* will probably live the longest, due (possibly?) to their stronger resistance to the lower temperatures.

5. The period of infectivity of the human intestinal protozoan cysts is not definitely known and more experiments should be made to determine it. It has been shown, however, that the cysts of *E. histolytica* and *E. coli* are infectious for man 2 and 10 days respectively, after their passage from the human body, and the cysts of *Giardia intestinalis* for kittens, after 74 days.

6. In the absence of more definite information regarding the period of infectivity, all prophylactic measures against the dissemination of protozoan diseases should aim to prevent the access of flies to feces and to destroy the feces within 2 days after their defecation.

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degenerative changes as those which occurred in stained cysts. This was true for all the different species of cysts.

It was first noticed that the nuclei became distinct, that the cytoplasm appeared more coarsely granular and that slight plasmolysis took place. Later on plasmolysis increased, and in the *Endamoeba coli* cysts the 3 characteristic zones developed as described above (Fig. 1). The final stages of degeneration were similar to those which occurred in the stained cysts. These degenerative changes took place in cysts which had been kept in a favorable environment (in a bottle containing distilled water) for several months, and at all times they refused to take up the eosin stain.

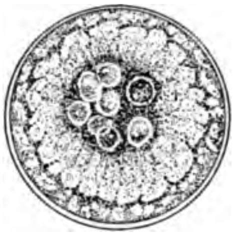

FIG. 1. *E. coli* cyst (dead). × 1860. Degeneration has resulted in the presence of the 3 zones in the cytoplasm.

But again, similar changes occurred within only a few days, when the cysts were in an unfavorable environment such as a stool not washed, or in cultures, in both of which there was an abundant bacterial growth resulting in the elaboration of putrefactive products of decomposition which hastened the degeneration of the cysts and led to the latter's early death.

From the work of Yoshida (1920) on the reproduction *in vitro* of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts, we note that many of the cysts which excysted had degenerated to a great extent and had plasmolized, for the cysts were described as possessing "a space, small or large . . . between the outer membrane and the cytoplasm," the dimensions of which varied greatly, "the largest observed being about half the size of the cysts." Certainly such changes were signs of degeneration similar to those observed in the cysts from the bottles, both those which stained with eosin, and those which remained refractive.

The question then arose, are all cysts alive which show marked degenerative changes and do not take up the eosin stain? This question is answered by making further reference to the experiments of

cystation in an artificial medium composed of agar and defibrinated horse blood, but the method was attended with unsatisfactory results as a whole, for only 3 of 11 experiments made on *E. tetragena* (*histolytica*) and only 7 of 19 on *E. coli* were successful. It was therefore inadvisable to use growth upon artificial media as a criterion of viability, since it was only indifferently successful in its application to the study of but two of the parasitic protozoa.

Another and valid criterion of viability which was used in the study of bacteria depended upon the ability of bacteria to produce an infection when inoculated into laboratory animals. This criterion could be employed to determine the viability of the cysts of *E. histolytica* and *Giardia intestinalis*, since infections in certain animals have been obtained with these organisms. It is possible to feed the cysts of *E. histolytica* to a kitten and obtain an infection, but not all attempts to produce an infection are successful. To obtain the desired results the cysts should be procured from a recent stool, and at the same time there should be a large supply of them, for with old cysts, and with a small number, the chances of obtaining an infection are very small. These conditions could not be met in this study, as only a limited supply of the cysts of this parasite were available.

Fantham and Porter (1916) produced an infection in mice and kittens by feeding them the cysts of *Giardia intestinalis*. But there is a grave danger in using these animals in testing the viability of the cysts of *Giardia intestinalis*, since they are often found parasitized by a species of Giardia, which does not differ materially from that living in man. Such a circumstance would lead to confusion unless proper precautions were taken, and these were too time-consuming to be practicable. But even if it were practicable to use the criterion of viability based upon animal infections, and satisfactorily determine the viability of cysts of *E. histolytica* and *Giardia intestinalis*, it would have had to be discarded on account of the fact that no infections have ever been obtained with *E. coli, Endolimax nana, Iodamoeba bütschlii,* and *Chilomastix mesnili*, the other species of cysts used in this study.

It was clear that neither of the above criteria of viability were practicable in their application to the study of all the different cysts, and this made it necessary to search for a criterion which could be uniformly utilized for all the cysts in the determination of their resistance and thermal-death point.

Kuenen and Swellengrebel (1913) were the first persons to use a dilute aqueous solution of eosin as a criterion of viability in the study of the cysts of *E. histolytica.* This stain is usually employed in the concentration of 1:1000. The above investigators held that this staining solution colored the dead cysts upon its penetration through the cyst wall, while the living cysts were not affected by the stain and remained very refractive in appearance. They also applied the test to the vegetative forms of *E. histolytica.*

Kuenen and Swellengrebel believed that the use of the eosin solution was a severe test in ascertaining the death of the cysts. They held to the rule that all stained cysts were dead, and that all the cysts which did not take the stain were viable. These investigators noted that the cytoplasm of the dead cysts became coarsely granular in appearance, and that it shrank away from the cyst wall for a short distance. When motile amoebae were killed and stained by the eosin solution, a dissolution of the cytoplasm took place, leaving the nucleus as the only remnant of the original organism.

After Wenyon and O'Connor (1917) had placed cysts of *E. histolytica* in boiling water and in strong disinfecting agents, and found that they stained pink in the eosin solution, while other cysts not similarly treated remained clear and refractive, they concluded ". . . that the eosin-stained cysts are dead, though it may be argued that others which do not stain may be dead also or, at any rate, non-infective."

Cutler (1920) added experimental proof in support of the eosin criterion of viability in his study upon the cysts of the soil protozoa. This investigator was able to bring about excystation of the cysts of the soil protozoa which were not stained by the eosin solution, but he was unable to do so with the cysts which had taken up the stain in their cytoplasm. Cutler's experiments were not sufficiently guarded from error, however, as to preclude the probability that a number of the unstained cysts, also, did not excyst, for the number of cysts at the beginning of the experiment was only estimated, and likewise the number of excysted amoebae. It is probable, then, that not all unstained cysts underwent excystation.

Yoshida (1920) was another investigator who employed the eosin-criterion of viability in his experiments on the reproduction in vitro of *Endamoeba tetragena (histolytica)* and *Endamoeba coli* from their cysts. He noted that sometimes the cysts did not take the stain, "although their nuclear and plasmic structures in stained preparations were shown to have degenerated or to have disappeared. . . . The living cyst is pale and markedly light-refracting, its cytoplasm is generally irregular, in large granules, and it appears rigid. The dead cysts which refract light slightly can be stained with eosin, while those which are markedly light-refracting do not take the stain." He concluded, as a result of his experiments, ". . . that cysts taking the eosin stain are dead, but not, on the other hand, that all dead cysts absorb this stain." This conclusion was based upon experimental data and substantiated the theoretical conclusion of Wenyon and O'Connor given above.

It seemed advisable, however, to use the eosin-criterion of viability in determining the thermal-death point of cysts of parasitic protozoa of man, since it could be applied with uniformity to the study of all the different species of cysts. Although it was shown by other workers, and also noticed in my own experiments, that some of the cysts seemed dead from the appearance of their plasmic alterations, but did not stain, such cysts were held as viable, for no exceptions were taken to the fast lines of distinction between things alive (unstained) and things dead (stained) which the eosin-criterion of viability imposes. The eosin-criterion may err—*i.e.*, not all dead cysts may take up the stain yet it is a safe criterion to adopt, for, as Wenyon and O'Connor stated, ". . . if we accept the eosin test as a criterion and regard all unstained cysts as living, the error in judgment will be on the safe side."

The observations and experiments upon all the species of cysts were made at Baltimore, excepting those on the cysts of *Iodamoeba bütschlii* and *Endolimax nana;* these were made at the Hygienic Laboratory of the United States Public Health Service, at Washington, D. C. I am indebted to Miss Katherine Flower for the preparation of some of the experiments upon these particular cysts. I also wish to thank Dr. Charles E. Simon for some of the *Giardia intestinalis* material used in this study.

THERMAL-DEATH POINT.

Technique.—From the work of Kuenen and Swellengrebel (1913), and also from the observations made upon the experiments by the author, it was apparent that the stools should be washed several times in order to obtain the cysts free from the great numbers of bacteria and the presence of the chemical products of decomposition in the feces, which exert a detrimental influence upon the vitality of the cysts. In one experiment with the cysts of *Endamoeba histolytica,* Kuenen and Swellengrebel (1913) showed that the cysts in one batch, which were kept in a glass of water in which there was a marked growth of bacteria, lived only 16 days, while the cysts in another batch, which were kept in another glass of water containing a sparse growth of bacteria, lived 29 days.

In some preliminary experiments by the author to determine the thermal-death point of the cysts of *E. histolytica,* the feces were not washed to secure the cysts free from the large numbers of bacteria and the putrefactive products, but simply broken up in a bottle of distilled water. In this bottle a pellicle soon appeared upon the surface of the water, which showed that the bacteria were not inhibited in their growth. Eighty per cent. of the cysts were dead (stained with eosin) 4 days after they had been immersed in the distilled water, and this was only 5 days after they had been passed in the stool. The thermal-death point was found to be at 54° C. in 5 different series of readings; this point, as it will be seen later, was 12° to 14° C. lower than that reached with cysts taken from the same fecal specimen, but which had been separated from the bacteria and the putrefactive substances present in the stool.

Similar results were obtained in experiments made with the cysts of *Iodamoeba bütschlii.* In one experiment the stool was not washed, but only a very small lump of feces containing the cysts was placed in each of the several test tubes along with a little distilled water and then subjected to different temperatures. The thermal-death point occurred at 54° C.; this was 12° C. lower than the thermal-death point obtained with cysts which had been washed free from the bacteria and chemical substances of putrefaction of the stool. In 2 other experiments the cysts were not washed free from the bacteria and putrefactive products in the feces, but were simply suspended with some of the feces in a bottle of distilled water, then comminuted and allowed to stand. The medium had a profuse bacterial growth and a pungent odor. Cysts taken from this supply also showed a lowered resistance, and the thermal-death point was at 56° C. in one series, and 54° C. in the other.

These experiments demonstrated that the presence of the bacteria and the chemical products of putrefaction of the stool affected the vitality of the cysts by lowering their resistance, which resulted in an abnormally low thermal-death point.

Accordingly in all subsequent attempts to secure the different cysts for the experiments on thermal-death point determinations the stool was comminuted in distilled water, strained through one or two layers of cheese cloth to catch the large particles in the feces, and then allowed to sediment out in a bottle. The cysts fell to the bottom of the bottle along with the finer debris. The supernatant fluid was drawn off and new distilled water added. The sediment was then shaken up into a suspension again and allowed to settle a second time and the supernatant fluid again withdrawn. The process was repeated until the supernatant fluid was clear, which in the early washings was colored and contained large numbers of bacteria and the soluble putrefactive substances. The cysts were transferred with the sediment to another bottle which contained about 50 to 100 c.c. of distilled water, and kept in a cool place where the temperature varied from 12° to 22° C. This low temperature inhibited the excessive proliferation of the bacteria in the bottle, for at no time was a pellicle seen upon the surface of the water, nor was any odor ever detected emanating from the bottle to indicate rapid putrefaction. In these supply bottles the cysts were found in the fine superficial layer of silt which had precipitated after the coarser debris had first settled to the bottom.

To determine their resistance to various temperatures and the thermal-death point, the cysts were withdrawn with a little sediment and water by means of a pipette and then placed in small test tubes of the size employed in the Wassermann test. The sediment and water containing the cysts in each tube comprised about 0.5 c.c. in volume. The cysts in the test tube were placed in a water bath after a given temperature had been reached and kept there for 5 minutes. Precautions were always taken to maintain the proper temperature for the entire 5 minutes.

In the experiments to determine the thermal-death point of any of the different cysts, the cysts were subjected to temperatures having an interval of 2° C. between them. This small interval made it possible to study the resistance of the cysts to gradual increases in temperature and also the determination of the number of dead (stained) cysts at these temperatures. After their exposure, some of the cysts were taken from the test tubes and examined upon the same day or allowed to wait until the following day, in order to ascertain how many were dead. No marked difference was noticeable in the results of the examinations made upon the cysts the same day that they had been exposed to the temperature, and the results of the examinations of the cysts made on the following day. The thermal-death point was the same in most cases.

Examinations.—The examination of the cysts was as follows: A drop of the sediment containing the cysts was taken from each of the test tubes by means of a pipette and deposited upon a glass slide. There it was rubbed up in a small drop of the eosin solution (a 1 per cent. concentration was used in these investigations). A coverslip was then placed upon this stained wet-preparation. In the examination of the stained wet-preparation, a "reading" constituted the counting of the first 50 cysts observed, and recording the number out of these which stained (dead). Therefore the figure recorded in the tables to follow under each interval of temperature always represents the number of stained cysts found in the first 50 cysts encountered. These stained cysts had died as a result of their exposure to the different temperatures. A control reading was made for each series. This reading was made upon cysts which had not been subjected to any heat previous to their examination. They were taken from the same supply bottle as were the cysts which had been exposed to the high temperatures in the determination of the thermal-death point.

A neutral red solution was also tried instead of the eosin solution as a test of viability, but it had no advantage over the latter. Root (1921) used the neutral red solution to determine the period of survival of the cysts in the gut of the fly, and found that 2 to 4 per cent. more cysts stained by the neutral red than by the eosin, and he concluded that the neutral red test was a little more delicate compared to the eosin criterion. A difference, however, of 2 to 4 per cent. means only 1 or 2 additional stained cysts, since only the number of living cysts were recorded out of the first 50 cysts encountered, and a variability of this slight extent very often occurred between 2 successive readings for any interval, when using either neutral red or the eosin solution. It seems likely, therefore, that the difference of 2 to 4 per cent. might well be included in the scope of experimental error, and that solutions of neutral red and eosin are equally efficient as indicators of death.

Definition.—Several series of readings were made for the cysts of the different parasitic protozoa, and in most cases the temperature at which all the cysts were found stained by the eosin, in the majority of the series, was considered the thermal-death point. As a rule it was found that the different series of readings of any one species of cysts did not vary materially from each other, and that the thermal-death point was generally constant within 2° C.

The thermal-death point was determined for the cysts of the following parasitic intestinal protozoa of man: *Endamoeba histolytica, Endamoeba coli, Endolimax nana, Iodamoeba bütschlii, Giardia intestinalis* and *Chilomastix mesnili.**

Endamoeba histolytica cysts.

The cysts of *E. histolytica* were obtained from two sources. The cysts used in the first 4 series of readings (Table 1) to determine the thermal-death point were passed in a stool on December 8, 1919, by a patient who was undergoing treatment for amoebic dysentery. The cysts which were used in the last 4 series of readings came from the feces of a healthy carrier on December 14, a year later. The cysts from both sources did not differ in proportion, both belonging to the same size race. When unstained they measured from 9 to 12 microns in diameter; those with a diameter of 10 to 11 microns predominated. The cysts from the first supply contained no chromatoidal bodies. A small minority of the cysts of the second supply showed these bodies to be feebly developed. In both supplies the cysts contained either 1, 2 or 4 nuclei; the 4-nucleated cysts were the most numerous. The cysts containing 1, 2 or 4 nuclei appeared equally susceptible to the high temperatures.

TABLE 1.

Series of readings to show the thermal-death point of the cysts of E. histolytica at 64° to 68° C.

No. of Series.	Date of Series.	Cysts passed.	Control.	48°	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	Date Examined
1	Jan. 28, 1920	Dec. 8, 1919	1	12	11	12	18	22	38						Jan. 29, 1920
2	Jan. 29, 1920	Dec. 8, 1919	1						35	43	42	48	50		Jan. 29, 1920
3	Feb. 6, 1920	Dec. 8, 1919	3				28	40	47	46	48	50			Feb. 6, 1920
4	Feb. 10, 1920	Dec. 8, 1919	4					34	44	46	49	50			Feb. 10, 1920
5	Dec. 23, 1920	Dec. 14, 1920	5				19	30	32	41	42	45	48	50	Dec. 24, 1920
6	Dec. 23, 1920	Dec. 14, 1920	5				19	33	35	40	43	44	47	50	Dec. 24, 1920
7	Dec. 27, 1920	Dec. 14, 1920	8				25	29	38	41	45	47	49	50	Dec. 28, 1920
8	Dec. 27, 1920	Dec. 14, 1920	8				23	30	33	38	46	48	49	50	Dec. 28, 1920

The first 4 series of readings (Table 1) were made 6 to 8 weeks after the cysts had been passed with the feces. These cysts, therefore, had undergone some degeneration. The thermal-death point in these series occurred at either 64° or 66° C. A year later, beginning December 23, 1920, 4 more series were made with the cysts obtained from the feces of the healthy carrier. In these cysts little or no degeneration had taken place, for they were only somewhat over 1 week old when the first series was made. This is the 5th series in Table 1. The control showed only 5 dead cysts out of the first 50 counted. The 5th, 6th, 7th and 8th, all made from the second supply of cysts, showed the temperature of 68° C. to be the thermal-death point.

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* The nomenclature employed to designate the different human intestinal protozoa is that most current in use, and it was for this reason alone, that the above terms were thought advisable. Until an opinion is forthcoming from the International Commission on Zoological Nomenclature, it seems best to apply the names of more common usage to the parasites and not to complicate the problem further by giving other generic and specific terms.

The thermal-death point in the last 4 series was 2° C. higher than that found for the cysts used in the first 4 series of readings. The rise in the thermal-death point was probably due to the use of fresh cysts as compared to those used in the first 4 series. It was also accounted for by the fact that the last 4 series were made within a short time after the cysts were passed out of the human body, while the first 4 series occurred within 2 weeks of each other, but from one to two months after the passage of the cysts in the feces. Hence it would appear that in view of the fresh condition of the cysts of the last 4 series the thermal-death point of 68° C. was closer to the truth than that of 64° and 66° C. in the earlier readings. It is more than probable that the resistance of the cysts used in the first 4 series had been lowered by some outside factors, such as the presence of bacteria and putrefactive substances, which had collected in the supply bottle from which the cysts were taken for the experiments. Although the temperature (12° to 22° C.) was low and inhibited the rapid proliferation of the bacteria in the bottle, yet by the time the first 4 series of readings were made, which was 6 to 8 weeks after the cysts had been washed and placed in the bottle, it was probable that the bacteria had multiplied to such an extent that they and the products of putrefaction had accumulated to the detriment of the cysts. The lowered resistance in these cysts resulted in the lower thermal-death point.

An average series of readings (Table 2) was computed from the

TABLE 2.

An average series of the cysts of E. histolytica to show the increase in mortality with each increase in temperature.

	48°	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°
Cysts of *E. histolytica*...	12	11	12	22	31.1	37.7	42.1	45	47.4	48.6	50

8 series given in Table 1. At the lower temperatures, 48° to 52° C., which were 10° to 14° above that of the human body, the cysts were very resistant. At 52° C. only 12 cysts were stained out of the first 50 cysts observed in the examination, or 24 per cent. When the cysts were exposed to temperatures higher than 52° C., the number of dead cysts increased with each increase of temperature, until all of them were dead at the thermal-death point of 68° C.

Endamoeba coli cysts.

The supply of cysts of *Endamoeba coli* was obtained from the feces of a patient who resided in Mississippi; the specimen was received on January 15, 1920. The stool upon examination showed a great number of *E. coli* cysts which measured from 15 to 21 microns in diameter, and also a large number of motile *Amoeba limax*. On February 5, 1920, the stool was washed and the cysts were placed in a bottle with distilled water, in the same manner as in the preparation of the cysts of *E. histolytica*. At this time the motile forms of *Amoeba limax* had all encysted; the cysts were present in aggregates resembling clusters of grapes. These cysts were small, measuring about 9 microns in diameter, and possessed a single vesicular, visible and centrally located nucleus and a very rough and thick wall. On account of these characteristics they led to no confusion in the examination of the *E. coli* cysts. The cysts of *Amoeba limax* often remained alive after their subjection to a temperature of 76° C. for 5 minutes.

The first series of readings to determine the thermal-death point of the cysts of *E. coli* was made on February 10, 1920, when the cysts were more than 3 weeks old, and after their washing 5 days previously. The first series of readings (Table 3) ran from 52° to 70° C. and demonstrated that the thermal-death point lay beyond 70° C., since only 39 cysts were dead out of the first 50 observed. It also showed that the temperatures of 52° to 64° C. had very little effect upon the cysts, for the number dead remained about the same for the intervals between these limits. The cysts began to show marked susceptibility to only those temperatures above 64° C.

In most of the following series of readings the thermal-death point of the cysts of *E. coli* was at either 74° or 76° C. In the 6th and 7th series of February 25 and March 3, however, the thermal-death point fell to 72° C. As in the case of the cysts of *E. histolytica*, when a fall in the thermal-death point occurred, the decline was due in all probability to some outside influence, such as bacteria or chemical sub-

TABLE 3.

Series of readings to show the thermal-death point of the cysts of E. coli at 72° to 76° C.

No. of Series.	Date of Series.	Cysts passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°	Date Examined.
1	Feb. 10, 1920	Jan. 15, 1920	7	7	12	10	13	14	13	14	14	—	19	39				Feb. 10, 1920
2	Feb. 12, 1920	"	5									19	18	41	45	45	50	Feb. 12, 1920
3	Feb. 17, 1920	"	4									—	26	41	49	50		Feb. 18, 1920
4	Feb. 18, 1920	"	4									27	35	45	49	50		Feb. 19, 1920
5	Feb. 20, 1920	"	4									35	46	47	49	50		Feb. 21, 1920
6	Feb. 25, 1920	"	8									31	40	43	50			Feb. 25, 1920
7	Mar. 3, 1920	"	24									45	45	49	50			Mar. 4, 1920
8	Mar. 8, 1920	"	19									37	—	42	46	49	50	Mar. 9, 1920
9	Apr. 14, 1920	"	12									38	39	—	41	48	50	Apr. 15, 1920

8 series given in Table 1. At the lower temperatures, 48° to 52° C., which were 10° to 14° above that of the human body, the cysts were very resistant. At 52° C. only 12 cysts were stained out of the first 50 cysts observed in the examination, or 24 per cent. When the cysts were exposed to temperatures higher than 52° C., the number of dead cysts increased with each increase of temperature, until all of them were dead at the thermal-death point of 68° C.

Endamoeba coli cysts.

The supply of cysts of *Endamoeba coli* was obtained from the feces of a patient who resided in Mississippi; the specimen was received on January 15, 1920. The stool upon examination showed a great number of *E. coli* cysts which measured from 15 to 21 microns in diameter, and also a large number of motile *Amoeba limax.* On February 5, 1920, the stool was washed and the cysts were placed in a bottle with distilled water, in the same manner as in the preparation of the cysts of *E. histolytica.* At this time the motile forms of *Amoeba limax* had all encysted; the cysts were present in aggregates resembling clusters of grapes. These cysts were small, measuring about 9 microns in diameter, and possessed a single vesicular, visible and centrally located nucleus and a very rough and thick wall. On account of these characteristics they led to no confusion in the examination of the *E. coli* cysts. The cysts of *Amoeba limax* often remained alive after their subjection to a temperature of 76° C. for 5 minutes.

The first series of readings to determine the thermal-death point of the cysts of *E. coli* was made on February 10, 1920, when the cysts were more than 3 weeks old, and after their washing 5 days previously. The first series of readings (Table 3) ran from 52° to 70° C. and demonstrated that the thermal-death point lay beyond 70° C., since only 39 cysts were dead out of the first 50 observed. It also showed that the temperatures of 52° to 64° C. had very little effect upon the cysts, for the number dead remained about the same for the intervals between these limits. The cysts began to show marked susceptibility to only those temperatures above 64° C.

In most of the following series of readings the thermal-death point of the cysts of *E. coli* was at either 74° or 76° C. In the 6th and 7th series of February 25 and March 3, however, the thermal-death point fell to 72° C. As in the case of the cysts of *E. histolytica,* when a fall in the thermal-death point occurred, the decline was due in all probability to some outside influence, such as bacteria or chemical sub-

TABLE 3.

Series of readings to show the thermal-death point of the cysts of E. coli at 72° to 76° C.

No. of Series.	Date of Series.	Cysts passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°	Date Examined.
1	Feb. 10, 1920	Jan. 15, 1920	7	7	12	10	13	14	13	14	14	—	19	39				Feb. 10, 1920
2	Feb. 12, 1920	"	5									19	18	41	45	45	50	Feb. 12, 1920
3	Feb. 17, 1920	"	4									—	26	41	49	50		Feb. 18, 1920
4	Feb. 18, 1920	"	4									27	35	45	49	50		Feb. 19, 1920
5	Feb. 20, 1920	"	4									35	46	47	49	50		Feb. 21, 1920
6	Feb. 25, 1920	"	8									31	40	43	50			Feb. 25, 1920
7	Mar. 3, 1920	"	24									45	45	49	50			Mar. 4, 1920
8	Mar. 8, 1920	"	19									37	—	42	46	49	50	Mar. 9, 1920
9	Apr. 14, 1920	"	12									38	39	—	41	48	50	Apr. 15, 1920

stances, which lowered the resistance of the cysts and caused their early death. A part of the proof for this statement is based upon the experimental data given above, showing how the presence of large numbers of bacteria and the products of putrefaction resulted in a lower thermal-death point of the cysts of *E. histolytica* and *Iodamoeba bütschlii,* and also upon the fact that this outside influence which increased the rate of mortality and lowered the thermal-death point of the cysts of *E. coli* on February 25 and March 3 was only temporary, for the thermal-death point was again back at 76° C. on March 8. The resistance of the cysts was only lowered for the brief period from February 25 to March 8, after which time those cysts which did not succumb regained their original power of resistance.

More proof of the temporary presence of this influence may be seen by a study of the controls of the different series. In series 1, February 10 (Table 3), 7 cysts were dead in the control, and this number decreased to 5 in the 2d series on February 12, and again to 4 in the 3d series on February 17, at which number it remained until February 25. On February 25, in series 6, the number of dead cysts in the controls increased to 8, and later in series 7, on March 3, to 24; after which a decrease ensued, with 19 cysts dead on March 8, and only 12 in the last series on April 14, 1920. The period when the outside influence was present in the bottle which lowered the resistance of the cysts and their thermal-death point coincides exactly with the period when a large number of cysts were dead in the control. This fact was proof that the increased death rate among the cysts was due directly to this outside temporary influence.

The reason the controls of March 3, March 8, and April 14 did not show a larger number of dead cysts than they did, since the examination upon these dates was made after the period of an increased rate of mortality, was due to the disappearance of the stained cysts which had died some time previously, through plasmolysis and disintegration. On these dates many empty cysts were found in the course of the examination.

It was found that in a supply of cysts in which there was an increased death rate, which had been preceded and followed by a slower one, that the small number of dead cysts which occurred as a result of a slow death rate at once became much larger in the period characterized by an increase in the rate of mortality, and that after this latter period of a rapid death rate the number of cysts became less again as a direct result of two factors—the resumption of a slow rate of mortality and the disappearance of the dead cysts through plasmolysis and disintegration. Had the dead cysts, which died during the previous period of a rapid death rate, not disappeared, then the number of dead cysts would have been larger when the slow death rate was resumed again.

TABLE 4.

An average series of readings of the cysts of E. coli to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	74°	76°
Cysts of *E. coli*....	7	12	10	13	14	13	14	14	33	33.5	43.3	47.3	48.6	50

In Table 4 are the figures of a series of readings for the cysts of *E. coli,* which represent an average of the 9 series in Table 3. This average series of readings shows the high resistance of the *E. coli* cysts at temperatures ranging from 52° to 64° C. At 64° C. 94 per cent. of the cysts of *E. histolytica* had succumbed to the heat, but Table 4 shows that only 28 per cent. of the *E. coli* cysts died after exposure to this temperature. The cysts of *E. coli* were less susceptible to the high temperatures than the cysts of *E. histolytica.* The thermal-death point at 76° C. is at least 6 degrees higher than that of *E. histolytica* cysts.

Iodamoeba bütschlii cysts.

The cysts of this parasitic amoeba were previously known as "I"-cysts, because they usually show one, and sometimes two, deep brown colored masses in the cytoplasm, in the presence of an iodin solution. The brown masses are composed of glycogen and each is situated in a vacuole. The glycogen disappears in the course of the technique used with a permanent stain, such as iron-hematoxylin, leaving the vacuole empty. In 1919 Dobell created the genus *Iodamoeba* for this parasite and the specific name *bütschlii* was retained from an earlier description by Prowazek in 1912, of what is perhaps the same parasite, and which was then called *Entamoeba bütschlii.* Dobell (1919) believed that Prowazek's *Entamoeba bütschlii* was identical with the motile organism of the "I"-cysts and now known as *Iodamoeba bütschlii* (Prowazek 1912 emend).

The cysts are very irregularly shaped and measure from 8 to 20 microns in diameter. The cysts most often encountered measured from 10 to 15 microns. They generally have only a single nucleus, but occasionally a 2-nucleated cyst may be found. The nucleus is invisible in an unstained preparation, but when the cysts are stained with a solution of iodin the nucleus becomes visible with a more or less refractive membrane and a large, brightly refractive and eccentrically situated karyosome. The latter appears to be lodged upon the membrane. In preparations stained with iron hematoxylin the large karyosome does not lie directly upon the nuclear membrane, and peripheral chromatin may be seen lying in the space between it and the nuclear membrane. The peripheral chromatin may often be seen to be agglomerated into a crescentic mass such as was first noticed by Brug (1919). The cytoplasm contains large refractive granules; these may be seen both in the unstained cysts and those stained with the solution of iodin.

Five series of readings were made to determine the thermal-death point of the cysts of *Iodamoeba bütschlii*. The cysts were all obtained from a single specimen of feces which was passed on December 15, 1920.

TABLE 5.

Series of readings to show the thermal-death point of the cysts of Iodamoeba bütschlii, at 62° to 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Dec. 16, 1920	Dec. 15, 1920	0	4	8	9	21	45	48	49	50	Dec. 17, 1920
2	Dec. 19, 1920	"	0			16	42	47	49	50		Dec. 20, 1920
3	Dec. 19, 1920	"	0			17	44	45	49	50		Dec. 20, 1920
4	Dec. 21, 1920	"	0			11	24	43	46	49	50	Dec. 22, 1920
5	Dec. 21, 1920	"	0			12	22	38	46	48	50	Dec. 22, 1920

The 1st, 4th and 5th series of readings gave the thermal-death point at 64° C., while the 2d and 3d series showed that all the cysts had died at 62° C. (Table 5). The thermal-death point of 62° to 64° C. for the cysts of *Iodamoeba bütschlii* was lower than that of *E. histolytica* and *E. coli*. No doubt, therefore, the cysts are not so resistant as those of the other parasitic amoebae. But at the lower temperatures of 50° to 54° C. the cysts were strongly resistant to the heat. A great increase in the number of dead cysts occurred at 56° C. (Tables 5 and 6) over the number of dead at 54° C. Less than one half as many dead cysts were found at 54° C. than were found at

56° C. The number of dead cysts was gradually augmented at the subsequent temperatures below the thermal-death point.

In the examinations of the control of each series of readings no stained cysts were seen, but the cysts showed other changes. It was noticed that a gradual decrease in the amount of glycogen had taken place. On December 16 all the cysts had glycogen in their vacuole, but on December 22 only a small number of the cysts had the vacuole completely filled, while most of the cysts had either an empty vacuole or the vacuole contained less glycogen than originally, as it did not stain so deeply in the presence of the iodin solution.

TABLE 6.

An average series of readings of the cysts of Iodamoeba bütschlii to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *Iodamoeba bütschlii*	4	8	13	30.6	43.6	47.6	49.2	50

An average series of readings is given in Table 6; it was derived from the 5 series of readings given in Table 5. The larger number of cysts died after their exposure to the temperatures above 56° C. The thermal-death point was at 64° C. The cysts of *Iodamoeba bütschlii* might be expected to remain viable for a long period of time in a bottle with distilled water, if kept at a temperature from 12° to 22° C., for they were very resistant to temperatures below 54° C.; after 5 minutes' exposure to the latter temperature, only 18 per cent. of the cysts were dead. The great increase in mortality occurred at 56° C., when more than twice as many cysts died from exposure than at 54° C.

Endolimax nana cysts.

The *Endolimax nana* cysts were first described by Wenyon and O'Connor (1917) under the name of *Entamoeba nana,* but Brug (1919) showed that this organism should be classified under a new genus, since it differed greatly from the members of the genus *Entamoeba;* he accordingly assigned it to the genus *Endolimax* and retained the specific name of *nana.* Lately Dobell (1919) has added considerably to our knowledge of this parasite of man by describing the motile organism.

The cysts of *Endolimax nana* are generally ovoid in shape and measure from 8 to 10 microns in length (Dobell, 1919). They do not show any internal structure in unstained preparations, except for the presence of a few refractive granules. In iodin solution the cytoplasm usually stains yellow, and not brown, as is generally the case with the other species of amoebic cysts. The nuclei are very small and indistinctly visible. Each nucleus contains a karyosome which may appear brownish when viewed in one focal plane, and refractive when seen in another. The karyosome is eccentrically situated in a clear area and upon the nuclear membrane. The nuclei are from 1 to 4 (sometimes 8) in number. There is no peripheral chromatin. The nuclear membrane is generally indistinct in specimens stained with iodin, but visible in those well decolorized after staining by iron-haematoxylin. The presence of the small granules which in iodin solution appear brownish and refractive, depending as in the case of the karyosome upon the particular focal plane in which they are viewed, may be confused with the nuclei at first sight, but these granules are not situated in a clear area as are the nuclei. Dobell (1920) claims that the cysts do not contain any vacuoles or chromatoid bodies. Cysts containing glycogen, however, were encountered on rare occasions, but it was not clear whether the glycogen was situated in a vacuole, as in the cysts of *Iodamoeba bütschlii,* or localized within the cytoplasm itself, as in *Giardia* cysts. The glycogen body, accordingly, was not so definitely set off in the cytoplasm as in the cysts of *Iodamoeba bütschlii.* Cysts have also been seen which contained small bodies of varied shapes which stained like chromatin. It is possible that they represented chromatoid bodies, but again they may have been inclusions of another nature.

The cysts used in the experiments to determine their thermal-death point were characterized by the description given above, except that the size was more variable. The cysts measured from 6 to 12 microns in their greatest diameter. They were both ovoid and spheroidal in shape. In all other respects it may be said that they answered to the description given by Dobell (1920, pl. 2, fig. 10). The stool containing the cysts of *Endolimax nana* was passed on February 4, 1921.

Eight series of readings (Table 7) were made to determine the thermal-death point of this species of cyst. With the exception of the first series of readings, the thermal-death point occurred at 64° C. A fair degree of uniformity existed in the number of dead cysts for each interval of temperature in all the series of readings. There were two striking features of the 8 series. The first was the remarkably

TABLE 7.

Series of readings to show the thermal-death point of the cysts of Endolimax nana, at 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Feb. 7, 1921	Feb. 4, 1921	0			10	13	20	35	50		Feb. 8, 1921
2	"	"	0			7	14	19	37	49	50	"
3	Feb. 8, 1921	"	0	1	6	6	9	18	36	47	50	Feb. 9, 1921
4	"	"	0	3	4	5	10	20	40	45	50	"
5	Feb. 9, 1921	"	3			7	13	19	36	49	50	Feb. 10, 1921
6	"	"	3			9	12	17	38	45	50	"
7	"	"	3			6	14	18	32	46	50	"
8	"	"	3			10	13	18	38	46	50	"

small number of dead cysts which occurred at 50° C., for only 4 per cent. of the cysts were found stained. These cysts proved to be the most resistant of all species at this particular interval. The other striking feature of the series of readings was the great increase in mortality which occurred at 60° C. over that of the preceding interval of temperature. In nearly all the series there were twice as many dead cysts at 60° C. than at 58° C. This is shown in Table 8, where an average of 18.6 cysts were dead at 58° C., while an average of 36.5 cysts were dead at 60° C.

TABLE 8.

An average series of readings of the cysts of Endolimax nana to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *Endolimax nana* . .	2	5	7.5	12.2	18.6	36.5	47.1	50

The average series of readings in Table 8 was made from the 8 series given in Table 7. This series showed how remarkably resistant the cysts of *Endolimax nana* were to the temperatures of 50° C., but how there was a gradual increase in the number of dead cysts with each increase of temperature up to 60° C. At this latter temperature, however, a very marked increase of 36 per cent. occurred in the number of dead cysts. An increase of 21 per cent. took place at the next interval of 62° C., which was the same as that determined for the cysts of *Iodamoeba bütschlii* (Table 6).

On account of the similarity in morphology, except for size, of their nuclei, the cysts of *Iodamoeba bütschlii* and *Endolimax nana* may appear to belong to a single genus. Aside, however, from the other differences, purely in morphology, which is the reason for consigning these cysts to two different genera and species, is the evidence obtained in the experiments to determine their respective thermal-death point.

If we compare the series of readings for the cysts of *Iodamoeba bütschlii* with those for *Endolimax nana*, there are at once certain points of similarity, as well as others which are different, for each species of cyst. It will be seen from Tables 6 and 8 that the thermal-death point of both the cysts of *Iodamoeba bütschlii* and *Endolimax nana* was at 64° C. In this respect these two species of cysts are similar. This fact might be construed as evidence in favor of these cysts belonging to the same genus and species were it not for the additional fact that the readings differed from each other for the temperatures below the thermal-death point. The cysts of *Endolimax nana* were more resistant than those of *Iodamoeba bütschlii* at a temperature of 50° C., and whereas the great increase in mortality occurred at 56° C. (Table 6) for the cysts of *Iodamoeba bütschlii*, a similar increase in mortality occurred at 60° C. (Table 8) for the cysts of *Endolimax nana*. It was apparent from this study that the resistance of these two species of cysts was not the same when subjected to temperatures between 50° C. and 64° C., and the presence of this fact along with the differences in morphology does not seem to warrant placing these cysts in the same genus and species at the present time. Later, if it is shown that these organisms are but different size races of the same genus and species, or are otherwise generically related, then the differences in their resistance to high temperatures must receive a new interpretation, possibly from a physiological viewpoint, than that given above which appears as evidence in favor of separating these forms both as to genus and species.

Giardia intestinalis cysts.

The cysts of *Giardia intestinalis* which were used in the experiments to determine their thermal-death point were obtained from the feces of three different persons infected with this parasite. The cysts were ovoid in shape and varied in length from 10 to 15 microns and from 6 to 9 microns in width. Some of the cysts contained 2 and others 4 nuclei.

TABLE 9.

Series of readings to show the thermal-death point of the cysts of Giardia intestinalis, at 60° to 64° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	Date Examined.
1	Feb. 10, 1920	Feb. 7, 1920	20	25	37	41	48	48	50			Feb. 10, 1920
2	Feb. 20, 1920	Feb. 19, 1920	0			45	46	48	48	49	50	Feb. 20, 1920
3	Feb. 21, 1920	"	7	21	41	45	46	48	48	50		Feb. 21, 1920
4	Feb. 25, 1920	"	22		41	46	47	49	49	50		Feb. 25, 1920
5	Mar. 1, 1920	"	19		45	45	46	46	48	49	50	Mar. 1, 1920
6	Mar. 3, 1920	"	23		43	46	47	47	49	50		Mar. 3, 1920
7	Mar. 15, 1920	Mar. 13, 1920	30		44	46	47	46	49	50		Mar. 16, 1920
8	Mar. 22, 1920	"	34		45	47	47	48	50			Mar. 22, 1920

Only 1 series of readings was made with the cysts taken from the first supply of cysts of February 7, 1920 (Table 9). In this series the control showed 20 cysts dead out of the first 50 cysts counted and the thermal-death point was at 60° C. The next supply of cysts of February 19 was a healthier lot, for the control showed that all the cysts were unstained. The second series in Table 5, which is the first series made from this supply of cysts, gave the thermal-death point at 64° C. This was 4° higher than that of the first series. The next four series (Table 9) were also made with the cysts from the second supply. The last two series of readings in Table 9 were made with the cysts taken from the third supply of March 30, 1920. Sixty per cent. of these cysts were dead when the first series of readings was made on March 15. But the resistance of the remaining viable cysts had not been altered, for the thermal-death point of this series was at 62° C., while in the next series it was 2° lower.

TABLE 10.

An average series of readings of the cysts of Giardia intestinalis to show the increase in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°
Cysts of *G. intestinalis*.....	23	42.2	45.1	46.7	47.5	48.9	49.6	50

While the thermal-death point varied from 60° to 64° C., and although in the majority of the series it was at 62° C., it is more than probable that, providing the cysts are all viable to begin with, as in the second series of February 19, that the thermal-death point is 64° C. This thermal-death point is the same as that of *Iodamoeba bütschlii* and *Endolimax nana* (Tables 6 and 8), but lower than that of *E. histolytica* and *E. coli.*

As was done with the series of readings of the other species of cysts above, an average series of readings was also made for the Giardia cysts from the 8 series found in Table 9. The Giardia cysts were not very resistant to the lower temperatures, for at 52° C. 84.4 per cent of the cysts were dead. Two degrees lower, viz., at 50° C., they were much more resistant, since only 46 per cent. of the cysts stained with the eosin. The Giardia cysts were the least resistant to the high temperatures of all the different species.

Chilomastix mesnili cysts.

The cysts of *Chilomastix mesnili* are the smallest of all the cysts of the different intestinal protozoa of man. They are lemon shaped and measure from 4.5 to 6 microns in width and from 7.6 to 9 microns in length. In an unstained wet-preparation they appear refractive and show no internal structure, save the presence of 3 or 4 very bright spots and a dull area to one side of the cysts, which, with the aid of the oil immersion, may be identified as the nucleus. The cyst wall is uniform in thickness except at the somewhat pointed end of the cyst, where it is slightly thickened.

The cysts of *Chilomastix* which were used in the tests to determine the thermal-death point came from the feces of a patient who has

TABLE 11.

Series of readings to show the thermal-death point of the cysts of Chilomastix mesnili, at 68° to 72° C.

No. of Series.	Date of Series.	Cysts Passed.	Control.	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°	Date Examined.
1	Jan. 6, 1920	Jan. 2, 1920	0	5	4	9	8	12	13	15	29	47	50			Jan. 6, 192
2	Jan. 15, 1920	"	1						11	15	28	44	50			Jan. 16, 192
3	Jan. 28, 1920	Jan. 27, 1920	0						11	43	48	49	50			Jan. 29, 192
4	Feb. 6, 1920	"	0						5	8	26	48	50			Feb. 6, 192
5	Feb. 10, 1920	"	1						2	6	27	40	43	50		Feb. 10, 192
6	Feb. 17, 1920	"	0						6	8	15	—	41	50		Feb. 18, 192
7	Feb. 19, 1920	"	2						6	9	10	39	47	50		Feb. 19, 192
8	Mar. 1, 1920	"	1						17	22	24	22	39	47	50	Mar. 2, 192
9	Mar. 8, 1920	"	0						15	29	36	40	40	49	50	Mar. 9, 192
10	Apr. 14, 1920	"	2								36	38	48	49	50	Apr. 14, 192

carried the infection for more than two years. Only 2 batches of cysts were employed in securing the 10 series of readings given in Table 11.

The first and second series of readings of January 6 and 15, respectively, were made with the cysts of the first supply. The thermal-death point was 68° C. in both of these series. A remarkable resistance was shown to the temperatures from 50° to 60° C. when compared to that of the cysts of the other parasites.

The last 8 series of readings in Table 11 were made with the cysts from the second supply of January 27, 1920. The 3d series of readings in Table 11, or the first series made from the second supply of cysts, showed more cysts dead from exposure to the temperatures 62° to 64° C. than that which occurred at the same temperatures in any of the preceding or subsequent series. The cause of this increase in the death rate could not be learned or explained. The thermal-death point was at 68° C., as in the first two series of readings.

The next three series of readings of February 10, 17, and 19 showed a smaller number of cysts dead at 60°, 62°, 64° and 68° C. than in the earlier series, and the thermal-death point advanced 2 degrees to 70° C. Less than half a month later, on March 1, the thermal-death point advanced a second time; in this and the next 2 series of readings it was at 72° C.

It was impossible to find a satisfactory explanation for the advances of the thermal-death point of the cysts of *Chilomastix mesnili.* It is probable that the true thermal-death point is between 70° and 72° C., and that some factor which was present for a time in the supply bottle temporarily affected the resistance of the cysts, and at that time lowered the thermal-death point. With the disappearance of the influence, the cysts were gradually able to regain their normal resistance, establishing the thermal-death point at 72° C. in the last 3 series of readings.

This outside factor or influence was probably not bacterial and chemical in nature, as occurred in the preliminary experiments with the cysts of *E. histolytica* and *Iodamoeba bütschlii* which resulted in a decrease in the thermal-death point, as related above, for there were no indications of profuse bacterial growth in the supply bottle, nor was there any odor or pellicle present. The supernatant water was very clear.

The control column of readings in Table 11 presented striking results, for not more than 2 stained cysts were ever encountered.

This condition would lead one to believe that most of the cysts remained viable, and that the rate of mortality was practically nil. The cysts, however, were becoming scarcer, as was noticed from the length of time it required to find the 50 cysts in each preparation in each succeeding series. They were very few in number when the last series was made on April 14. This fact proved that the cysts were gradually dying in the supply bottle, from which the cysts were taken for the control readings. It was also apparent that the dead cysts were disappearing through disintegration in order to account for the small number of stained cysts encountered during the examination of the controls. Only a few cysts were observed which showed intermediate stages of plasmolysis in the control examinations and likewise a few empty cysts. The empty cysts, however, were so difficult of detection that undoubtedly they were often missed in the examinations. In view of this fact, the control readings can not be strictly correct. The death rate of the cysts in the supply bottle must have been very slow, since the temperature in the supply bottle was much lower than the temperatures of 50° to 62° C. in the experiments to determine the thermal-death point, and at which the cysts were so remarkably resistant.

TABLE 12.

An average series of readings of the cysts of Chilomastix mesnili to show the inincrease in mortality with each increase in temperature.

	50°	52°	54°	56°	58°	60°	62°	64°	66°	68°	70°	72°
Cysts of *Chilomastix mesnili*......	5	4	9	8	12	9.5	17.2	27.9	40.7	45.8	49.1	50

Table 12 gives an average series of readings which was computed from the 10 series given in Table 11. The series shows very clearly the evidence for the strong resistance of the Chilomastix cysts to the temperatures of 50° to 62° C., for at the latter temperature only 34.4 per cent. of the cysts were dead.

The number of dead cysts given at 60° C. is low, for 5 of the 9 readings given at this temperature in Table 9 showed 11 of 17 cysts were dead. The average reading at this temperature was lowered by the small number of dead cysts recorded in the readings of the 4th, 5th, 6th and 7th series. The thermal-death point in Table 10 is 72° C.

SUMMARY.

In the above experiments, with the aid of the eosin-criterion of viability, the thermal-death point of the different cysts of the parasitic intestinal protozoa of man was determined as follows: *Entamoeba histolytica,* 68° C.; *Entamoeba coli,* 76° C.; *Iodamoeba bütschlii,* 64° C.; *Endolimax nana,* 64° C.; *Giardia intestinalis,* 64° C., and *Chilomastix mesnili,* 72° C. In quoting the above temperatures as the thermal-death points, it will be remembered that they are subject to a slight downward deviation of generally 2° C., since all the experiments upon each species of cysts did not end in a common thermal-death point. The remarkable resistance of all the protozoan cysts to the high temperature is quite analogous to that of the spores of bacteria; in both cases the thermal-death point is much higher than that of their respective vegetative and non-spore bearing stage.

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WASKIA INTESTINALIS: ITS CULTIVATION AND CYST FORMATION[*]

MARY JANE HOGUE, Ph.D.
BALTIMORE

The small flagellate *Waskia intestinalis* was first described by Wenyon and O'Connor[1] in 1917. They found it in the stools of two patients in an army hospital in Alexandria, Egypt. They believed that the second patient became infected from the first patient, as the beds of the two patients were close together in the same ward. Both patients were admitted as carriers of *Endameba histolytica*. *Waskia* appeared in the first case a week after admission to the hospital, and withstood the injection of 12 grains of emetin. As the *Waskia* infection was disappearing, the patient developed a chilomastix infection. The second patient was being treated for *Endameba histolytica,* which was fast disappearing, when *Waskia* suddenly appeared in the stools three weeks after the patient had been admitted to the hospital. Both patients showed tendencies toward constipation and were treated with repeated doses of salts. Wenyon and O'Connor conclude that there is no evidence that the flagellate is pathogenic.

Kofoid, Kornhauser, and Plate[2] in 1919 reported *Waskia intestinalis* as occurring in seven patients in Debarkation Hospital No. 3 in New York City. Here it appeared in liquid diarrheic stools of patients under treatment. Three of the infections were from overseas men, and four from men in home service. Five of these cases were from the Southern states and two from New England. All were accompanied by multiple infections of from three to six other parasitic species.

Castellani and Chalmers[3] classify *Waskia intestinalis* under the order Cercomonadidae, family Tetramitidae, subfamily Embadomonadinae, genus Embadomonas.

Since these papers, *Waskia* has not been further reported. Public health officers have been much concerned with the possible spread of diseases brought over by returned soldiers. The following case of *Waskia intestinalis* infestation indicates that new protozoan diseases may be spreading here among the civilian population.

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[*] From the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University.

[1] Wenyon, C. M., and O'Connor, F. W.: Human Intestinal Protozoa in the Near East, London, Wellcome Bureau of Scientific Research, 1917.

[2] Kofoid, C. A.; Kornhauser, S. I., and Plate, J. T.: Intestinal Parasites in Overseas and Home Service Troops of the U. S. Army, J. A. M. A. **72**: 1721-1724 (June 14) 1919.

[3] Castellani and Chalmers; Manual of Tropical Medicine, 1919.

April 7, Dr. Milton Linthicum sent to this laboratory the stool of a patient who had a history of many years of diarrhea. She was known to have a trichomonas infection. She had never been out of the United States and had spent most of her time in Pennsylvania and Maryland.

From this specimen I inoculated several tubes of different kinds of mediums by means of a sterile platinum loop. The tubes were incubated at 35 C. In a few days, *Waskia intestinalis* and its cysts appeared in large numbers. In one tube I had a pure culture of *Waskia*, i. e., no other protozoa were growing with it, though there were numerous bacteria present from the intestine. This culture has been kept under continuous observation for over eight weeks. Transplants into new culture tubes are made every other day. The flagellates are still (June 6) in a normal, active condition.

This species seems to correspond with *Waskia intestinalis* as described by Wenyon and O'Connor. It is a small, active flagellate (Fig. 1), measuring in stained preparations from 5.6 to 7.2 microns in length and from 4 to 4.8 microns in width. There is a rather large cytostome and two anterior flagella. The one coming from the anterior end of the animal is long and slender, and is used for locomotion. The second flagellum comes from the cytostome and is shorter and much thicker. It seems to be used in getting food. The cytoplasm is mostly much vacuolated and contains many bacteria in food vacuoles.

In stained preparations the nucleus is seen in the extreme anterior region. It has a distinct membrane, with usually a small central karyosome. There are two blepharoplasts from which arise two flagella.

The cysts are pear shaped (Fig. 2), measuring from 4.8 to 7.2 microns in length and from 4 to 4.8 microns in width. They appear as small, light yellow, refractive bodies in the culture medium. Wenyon and O'Connor assumed that these cysts belong to *Waskia* because they found them only in the feces where *Waskia* was present. The appearance of these cysts in cultures where *Waskia* is the only organism growing, except the bacteria, is proof of their identity. As additional proof, some days there would be large numbers of *Waskia* in the cultures and on the following day the number would be greatly reduced and the number of cysts greatly increased. This seems to be the first time that cysts have been obtained from intestinal flagellates in culture, and proves beyond doubt that these cysts do belong to the flagellate *Waskia intestinalis.*

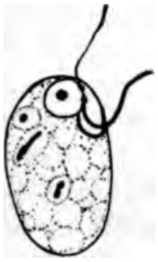

Fig. 1. — Waskia intestinalis, showing anterior nucleus and two anterior flagella, one much thicker than the other and coming out through the cystostome. Camera lucida drawing 1.9 mm. objective, 12.5 ocular.

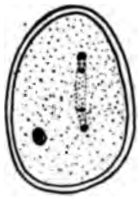

Fig. 2.—A cyst of Waskia intestinalis. Camera lucida drawing, 1.9 mm. objective, 12.5 ocular.

CULTURE MEDIUMS

Waskia intestinalis has been cultivated on three different liquid mediums: ovomucoid, Locke-egg, and an ox bile salt medium, and also on Boeck's medium of Locke with a few drops of sheep or human serum.

Ovomucoid medium is thus made: The white of one hen's egg is thoroughly shaken up with glass beads. To this 100 c.c. of 0.7 per cent. sodium chlorid solution is added. This is cooked for half an hour over a hot water bath; all the time the medium in the flask must be kept in constant motion by shaking. It is then filtered through cotton with a suction pump and about 6 c.c. of the filtrate put into small test tubes. To these sometimes are added a loop full of the yolk of the egg. The tubes are then autoclaved for twenty minutes under 15 pounds of pressure.

Locke-egg medium is made with one hen's egg thoroughly shaken up with glass beads. To this 200 c.c. of Locke solution is added. The Locke is made of sodium chlorid, 0.9 gm.; calcium chlorid, 0.024 gm.; potassium chlorid, 0.042 gm.; sodium bicarbonate, 0.02 gm., and sometimes 0.25 gm. of dextrose was added. This later did not seem to affect the growth of the organisms. The Locke and egg are cooked for fifteen minutes over a hot water bath and then filtered, tubed and autoclaved as the ovomucoid medium.

The ox bile salt medium contained 1 gm. of ox bile salts; 0.4 gm. of peptone, and 100 c.c. of 0.7 per cent. sodium chlorid solution. This was warmed to facilitate the solution of the salts and peptone. It was then tubed and autoclaved as the other mediums. Before using this medium, three or four drops of sheep or human serum[4] were usually added. On this medium *Waskia* divided most rapidly, but it did not live as long as on the other two mediums. On this ox bile salt medium there would be large numbers of flagellates on the second day, and these would usually disappear on the third day. A few cysts would be found, but there were not as many of these as there were organisms on the previous day, showing that many of the animals had disintegrated instead of encysting.

On the Locke-egg ovomucoid, *Waskia* lived much longer. One culture lived for seventeen days on ovomucoid. On the Locke-egg medium they lived from three to ten days. To both these mediums I sometimes added three or four drops of sheep serum or human serum. This often seemed to increase the rate of division and also to shorten the life of the culture, owing no doubt to the increased accumulation of waste products. In both the ovomucoid and in the Locke egg cysts were found. These would begin appearing about the fourth day after inoculation. Only a few cysts were found in the ox bile medium, as already stated. The Locke-egg is especially to be recommended, as the protozoa multiply rapidly and seem normal on it without the addition of serum, which is often difficult to obtain.

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[4] Mrs. Hogan of the Public Health Laboratories kindly gave me the serum from the Wassermann tests.

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THE CULTIVATION OF TRICHOMONAS HOMINIS

MARY JANE HOGUE

Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland

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There are many different species of Trichomonas, three of which have been described as parasitic in man. These are *Trichomonas buccalis*, Goodey and Wellings; *Trichomonas hominis*, Davaine; and *Trichomonas vaginalis*, Donné. It is even possible that these three species are the same but this has not yet been proved.

In 1915 Lynch cultured *Trichomonas hominis* from the intestine in neutral and acid bouillon and kept it alive for three days at 30°C. By transferring to new media he was able to carry the culture through five or six generations.

Ohira and Noguchi (1917) report the cultivation of the Trichomonas from the human mouth calling it *Tetratrichomonas hominis*. According to Kofoid's (1920) recent review of the nomenclature of the flagellates, Ohira and Noguchi were working with *Trichomonas buccalis*. They cultured it on a mixture of ascitic fluid and Ringer's solution, using equal portions and found that it gave much better results than Lynch's bouillon medium. They carried their strains for over twenty generations.

Boyd (1918) reports the cultivation of *Trichomonas hominis* on a non sterile medium which was a mixture of faecal material and physiological saline.

Pringault (1920) used Ohira and Noguchi's medium for the cultivation of *Trichomonas intestinalis*, i. e., hominis, very successfully.

Recently (1921) Reuling has cultured *Trichomonas vaginalis*. He tried many media but says the flagellates never lived longer than twenty-four hours on any of the media except that described by Ohira and Noguchi. (One-half Ringer's solution and one-half ascitic fluid). On this medium *Trichomonas vaginalis* divided, reaching its greatest period of reproduction on the fourth or fifth day. On the following day all the organsims had completely disappeared. He kept them at 37°C.

On April 7 a stool containing *Trichomonas hominis* was sent to this laboratory. Several test tubes of different kinds of media were inoculated with the material by means of a sterile platinum loop and incubated at 35°C. On May 4 a single individual was isolated from the general culture and used to start a "pure line" of Trichomonas. A very simple method was used to isolate the pure line. Small drops of the medium in which the Trichmonas were not very plentiful, i.e., young or, old cultures, were placed on cover slips which were then inverted over depression slides and examined. Trichomonas is plainly visible under the high dry objective. When the drop was found to contain only one Trichomonas the corners of the cover slip were cut off with scissors and the remaining small piece of glass with its single Trichomonas was dropped into a test tube containing Locke-egg medium. This was then incubated at 35°C. and in a few days the medium was swarming with Trichomonas. The pure line as well as the general stock culture are now (June 7) both in a normal condition after more than eight weeks of cultivation on sterile media. Transfers to new media have been made every second, sometimes every fourth day, depending on the general condition of the cultures. All cultures are kept in a constant temperature chamber at 35°C.

Many individuals in the pure line of Trichomonas have been carefully studied. They have four anterior flagella and an undulating membrane with a lateral marginal flagellum which leaves the membrane toward the posterior part of the animal. These cultured flagellates measure from 7 to 10μ in length and from 5 to 8μ in width. They are very active in the liquid media, moving by means of their flagella. The formation of pseudopodia was not observed.

MEDIA

A medium which is the combination of a hen's egg and Locke solution proved most successful for the cultivation of Trichomonas hominis. A hen's egg is thoroughly shaken up in a flask with glass beads. To this is added 200 cc. of Locke solution which is made in the following way.

	grams
Sodium chloride	0.9
Calcium chloride	0.024
Potassium chloride	0.042
Sodium carbonate	0.02
Dextrose	0.25
Distilled water	100 cc.

This was heated over a hot water bath and kept in constant motion for fifteen minutes. It was then filtered through cotton with a suction pump and about 6 cc. of the filtrate put into test tubes These were autoclaved for twenty minutes under fifteen pounds of pressure.

An ovomucoid medium was made from the whites of two eggs, which were thoroughly shaken up with glass beads. To this was added 200 cc. of 0.7 per cent sodium chloride solution. This was cooked for half an hour over a hot water bath and kept all the time in constant motion. It was then filtered, tubed and autoclaved just as the Locke-egg medium.

On the Locke-egg medium Trichomonas appeared in greatest numbers on the second day after inoculation. Then as many as 23 organisms could be counted in the field of a number 16 objective, 10 ocular. These were of all sizes, large ones preparing to divide and smaller ones which had already divided. This difference in size even in the pure line cultures was very noticeable when the Trichomonas appeared in great numbers at the height of their reproductive period. On this medium they rarely lived longer than six days.

On the ovomucoid medium they usually reached their greatest period of productivity on the third day. The cultures lived from six to ten days, a few lived 11 days. When a few drops of sheep serum or, better still, human serum were added the organism appeared in even greater numbers. At times from 40 to 45 Trichomonas could be counted in one field. These rich cultures also died more quickly, no doubt due to the accumulation of waste products.

Trichomonas did not encyst in any of the cultures. Nothing resembling a real cyst was ever found. They would become less active in the older cultures. Then they would round up and usually form a large vacuole. Soon they would go to pieces. Pringault describes the same process as taking place in his cultures. Perhaps these rounded organisms are the same as Lynch describes as cysts but I cannot consider them as such. They are of very short duration. One rarely finds them the day after the active Trichomonas have disappeared.

These media have proved so successful for the cultivation of intestinal protozoa that it seemed wise to share them with other investigators. The fact that neither ascitic fluid nor serum is necessary makes the media available for all investigators and teachers even though they are not connected with hospitals or public health laboratories.

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A COMPARISON OF AN AMOEBA, VAHLKAMPFIA PATUXENT, WITH TISSUE-CULTURE CELLS

MARY JANE HOGUE

Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, and the Department of Embryology of Carnegie Institution of Washington

THREE FIGURES

INTRODUCTION

While working with tissue cells grown in culture medium I was much impressed with the similarity of their appearance to that of amoebae grown on agar-agar plates, and determined to make a comparative study of these two kinds of cells. The particular cells used for this study were the fibroblasts and the white blood cells from the embryonic chick and an amoeba, Vahlkampfia patuxent (Hogue, '21), which is a salt-water form, parasitic in the digestive tract of the oyster.

The tissue cells were grown at 39° in hanging drops of Locke-Lewis solution (Lewis and Lewis, '15) inverted over hard vaselin rings on depression slides.[1] The amoebae were taken from agar-agar plates made up with 0.7 per cent sodium chloride solution, with 0.4 per cent peptone, and kept at room temperature.

The comparison of these two kinds of cells was made by studying permanently stained preparations, by introducing the amoebae into the cultures where the tissue cells were growing and here studying their different reactions to various vital stains and pigment granules and by further testing out the effect of these stains and pigment granules on the different kinds of cells in their own culture media.

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[1] Mrs. Lewis kindly furnished me with tissue cultures of connective tissue and spleen.

MORPHOLOGY AND MOVEMENT

At first the most noticeable difference between these cells is their diversity in size. The amoebae averaged 21 μ in length and were much smaller than the fibroblasts which measured 50 μ in length. The exact size of the fibroblasts is hard to determine, as their processes are so thin and fine that their farthest limits are difficult to determine. The amoebae always have a definite outline, though they are continually changing shape. Still another difference is that the amoebae can be observed to move rapidly, while the movement of the fibroblasts is too slow to be noticeable, though they do move as can be seen by watching the growth of the culture from day to day. The amoeba moves by lunging in one direction and then in another. The cytoplasm is thicker and denser than in the fibroblasts where the processes are very thin and flat against the cover-slip.

In order to study the two kinds of cells together, the amoebae and the bacteria growing with them were introduced by means of a platinum loop on the cover-slip where the tissue cells had been growing for from twenty-four to forty-eight hours. The amoebae began at once to crawl actively around and over the fibroblasts without apparently disturbing them.

The tissue cells had to be kept at 39°C. The amoebae ordinarily live at room temperature, though, as I have already shown (Hogue, '21), they can be kept at 35°C. for twenty-four hours, after which they showed a decided tendency to encyst some time during the following week, the encystment not following immediately on their removal from the high temperature. When the amoebae were brought into the warm box, where the cultures were examined microscopically, their rate of motion was greatly increased. They were now moving rapidly, and continued to do so as long as they were kept at this high temperature.

REACTION TO BACTERIA

The presence of the bacteria, which were chiefly non-pathogenic bacilli serving as food for the amoebae, was very harmful to the tissue cells. They evidently could not stand the toxic effect of the bacterial waste products. In a few instances some of the fibroblasts lived twenty-four hours after the introduction of the amoebae and the bacteria into the tissue culture, though most of the fibroblasts pulled in their processes and contracted into small oblong masses within a few hours after the introduction of the amoebae and bacteria. Later they went to pieces, while the amoebae moved about quite normally in this new medium (Locke-Lewis), and lived for over two weeks, when the experiment was discontinued.

REACTIONS TO STAINS

Neutral red

In trying out the effect of vital stains on the cells it was found that a very weak solution of neutral red was sufficient to give an excellent stain. When neutral red 1 to 10,000, the proportion used for tissue-culture cells, was used with the amoebae, they would immediately round up, stain red, and die. This solution was sometimes allowed to dry on a cover-slip, which was then put down on the drop of Locke or normal salt solution containing the amoebae. The neutral red would gradually diffuse into the medium and stain the neutral-red granules and vacuoles of the amoebae without immediately killing them. Experiments with neutral red of different strengths were made; 1 to 40,000, 1 to 60,000, and 1 to 80,000 in Locke solution were all good and not sufficiently strong to kill the animals. Amoebae were kept in hanging drops of Locke solution with neutral red 1 to 40,000 for nine days; at the end of this time they were moving slowly and had a few neutral-red granules. In another set of experiments the amoebae were kept in Locke solution with neutral red 1 to 80,000 for twenty-four hours. At the end of this time most of them had lost the stain, so Locke with neutral red 1 to 60,000 was added, and they continued active with stained neutral-red granules for seven more days, when the experiment was discontinued. This is especially interesting, as in tissue cultures the disappearance of the color from the neutral-red granules was always taken as an indication that the cells were dead. With the amoeba after the color has disappeared the granules can be restained with neutral red and the amoeba is still normally active.

The disappearance of the neutral red from the amoeba seems to be due to oxidation. One small amoeba was watched for five hours. At 9:00 in the morning it contained fifteen neutral-red granules. The color gradually faded from these granules until by 11:30 only one granule had the neutral-red color and by 12:00 this color had disappeared. The granules themselves did not dissolve, but could be still seen in the amoebae. Unfortunately, janus black no. 2 had been also used, and this eventually kills the cells. However, at 4:30, this particular amoeba was still very active with numerous stained mitochondria, but the next morning it was dead.

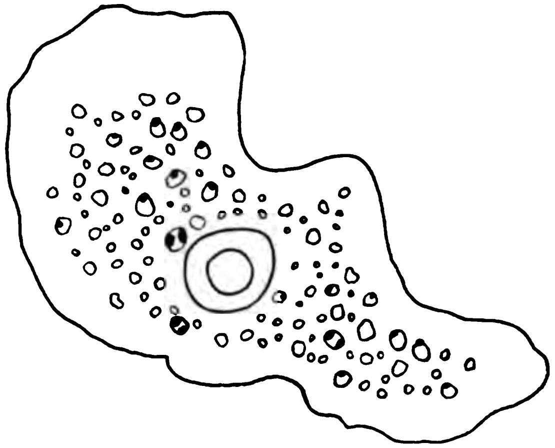


Fig. 1 Amoeba showing neutral-red granules of different sizes and neutral-red vacuoles containing one or two neutral-red granules. 12.5 ocular, 1.9 oil immersion.

The neutral-red granules and vacuoles were of varying size and number. Some amoebae had many small granules and many large vacuoles, which contained from one to two granules (fig. 1). In the fibroblasts one frequently finds neutral-red channels, but they have never been observed in the amoebae.

Brilliant cresyl blue 2 b

The amoebae were much more sensitive to this stain than were the tissue-culture cells. One drop of a weak solution was added to the tissue culture. The fibroblasts responded at once in the usual way (W. H. Lewis, '19) to this stain. Many of the vacuoles stained pink with one or more purple granules. The vacuoles were grouped around the nucleus and centriole, just like the neutral-red granules. This same solution applied to the amoebae stained the cytoplasm a pale blue, the granules a purple blue, and the karyosome of the nucleus a purple blue with a clear area in the center. The nucleus with its karyosome and clear area were pulled away from the endoplasm out into the ectoplasm. The amoeba moved slowly and sent out many large clear pseudopodia, a kind of lunging movement similar to blebs in tissue cells. Within half an hour the granular endoplasm had contracted into a solid blue mass of irregular shape, the nucleus remaining in the ectoplasm. Motion had stopped and the amoeba was dead.

This solution of brilliant cresyl blue was diluted one-half with Locke solution and applied to the amoebae. This time about half of the endoplasmic granules took the deep blue stain, the other half remained unstained and were quite refractile. The karyosome stained purple as before, but with a bluish purple area around it and the cytoplasm was a pale blue. The amoebae moved actively, but in a short time the granules lost their color and the nucleus and cytoplasm stained more deeply and the amoebae were dead.

When the solution was again diluted one-half and added to a new hanging drop of amoebae, the effect was the same as that on the tissue cells with a solution four times its strength. The nucleus was not stained. The endoplasm contained purple granules as well as the pink vacuoles with their purple granules. The amoebae moved rapidly and progressed normally.

Methylene blue

The solution of methylene blue, when added to the tissue cells, did not stain the nucleus nor cytoplasm. The vacuoles stained blue and the one or more granules within them took a deep blue stain. Blebs were forming on many of the cells. The same solution added to the amoebae stained the karyosome a deep blue, the granules a deep blue, and the cytoplasm blue. The nuclear area around the karyosome remained clear and the amoebae moved freely. Later they rounded up into blue balls surrounded by a lighter blue ectoplasmic area. In this area the nucleus was usually found away from the endoplasm. The amoebae were dead.

When the solution of methylene blue was diluted one-half, the effect on the amoebae was the same, though the animals did not die so quickly. When it was diluted one-fourth of the original strength, it stained the amoebae the same as the original solution had stained the tissue-culture cells. The cytoplasm was clear, the nucleus unstained and invisible except as a clear area in the endoplasm free from granules. Many of the granules were colored blue, though not all took the stain. To this stain also the amoeba cell is four times as sensitive as the tissue-culture cell.

MITOCHONDRIA

The amoebae were treated with janus black no. 2. A very weak solution was used, as otherwise the amoebae would contract and die without showing the mitochondria at all plainly, though the nucleus stained well.

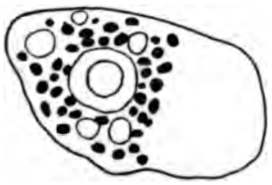
When the amoebae stained slowly the mitochondria appeared as small short rods, like bacilli, in among the neutral-red granules (fig. 2). Some of the mitochondria were round, and frequently after they had been stained for some time groups of three or four small mitochondria would be found in among the larger ones (fig. 3). No long branching mitochondria were found in the amoebae, though they are very common in the fibroblasts. The mitochondria retained the stain and were visible as long as the amoebae were alive. This stain seemed to have a stupefying effect, as the amoebae died with their pseudopodia extended instead of rolling up into balls.

The mitochondria, when the amoeba is dying, have very marked brownian movement. In the later stages of death the mitochondria move out into the clear ectoplasm which before has been free from granules of all kinds. This does not occur in the tissue-culture cells, as I have already shown (Hogue, '19).

REACTION TO MELANIN PIGMENT GRANULES

In studying the amoeba's reaction to melanin pigment granules, I first introduced the amoebae into tissue cultures of the spleen which had been growing with the melanin pigment from the retina of a chick embryo for from twenty-four to forty-eight hours. Later I simply put the granules into hanging drops of 0.7 per cent normal salt solution with the amoebae.

As soon as the amoebae recovered from being transferred and were moving about with their large ectoplasmic pseudopodia, they began to take in the pigment granules. The amoebae reacted differently to these granules which were in great numbers and were showing brownian movement. Some of the amoebae at once took in large numbers of the granules, others only a few, and still others moved about freely among the granules without taking in any of them. There was evidently some difference in the physiological condition of the amoebae at that time.



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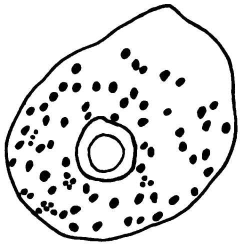

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Fig. 2 Amoeba stained with neutral red and janus black no. 2. The neutral-red granules are clear, the mitochondria are solid black. 12.5 ocular, 1.9 oil immersion.

Fig. 3 Amoeba stained with janus black no. 2. The mitochondria are oblong and a few small ones are arranged in groups. 12.5 ocular, 1.9 oil immersion.

Many of the granules were oblong, so that it was easy to determine their movement in the cells. They always entered by way of the anterior pseudopodium, whether it was one large pseudopodium or two smaller ones. The amoeba took in the granules in the same manner that it takes in its food. The granules had to be in the same plane as the advancing pseudopodium. If they were above it or below it the amoeba simply went past it without taking it in. The granules could lie in any position in relation to the advancing pseudopodium. They were horizontal and perpendicular to it and at times formed an angle with it. Often they were swung around from a perpendicular to a horizontal position and vice versa, and sometimes they swung from a perpendicular or horizontal position to a slanting one and vice versa as they entered the ectoplasm. Once in the ectoplasm their position was constant until they reached the endoplasm. As soon as they were there, they were whirled about by the endoplasmic currents.

As a rule, only one granule was taken in at a time, though I have seen a group of three go in at once, and frequently two granules will be taken in at different parts of the pseudopodium at the same time. The amoeba is capable of taking in a large number of these granules. Four hours after the pigment granules were introduced into the hanging drop of amoebae, the amoebae were filled with the granules. They had at least a hundred granules and resembled the pigment cells of the retina, except for the fact that they were moving rapidly.

The amoeba takes the granules in by simply pushing against the granules with such force that the granule pierces the ectoplasm. For this reason the granule to be taken in must be in the path of the advancing amoeba. If it is to one side of the anterior pseudopodium it is passed by; if it is near the side of the pseudopodium, but still anterior to it, it may be taken in or it may be pushed off farther to the side, depending on how far to the side it is and also on how rapidly the amoeba is moving. If it is moving very fast the granules near the outer edge of the anterior pseudopodium are much more likely to be pushed away than to be taken in, or they may remain attached to the amoeba like the spines of a sea-urchin and be passed back along the edge until they reach the posterior end. Here they frequently remain attached for a long time, but are never taken in at this point. When the amoebae have been in a medium full of these melanin granules for some time many of them are found carrying at least fifty or more granules on their pointed posterior ends. They fairly bristle with the pigment granules at this point.

After the granules have entered the endoplasm, they circulate freely through it. Sometimes two or more will unite and occasionally a vacuole will form about these fused granules. Sometimes there will be as many as three or four clumps of granules in one vacuole. These vacuoles are heavy and lag behind in the posterior part of the amoeba. Eventually they come in contact with the outer edge of the amoeba. After that they soon break, discharging the pigment granules to the outside. Some of the granules remain attached to the amoeba, others, more forcibly ejected, float away in the medium. In this way the amoeba gets rid of the melanin pigment. After twenty-four hours most of the amoebae had given off the granules and were moving about freely among the granules without taking them in again, at least not in such large quantities as when the granules were first introduced into the hanging drop. The amoebae never digested the melanin pigment.

Small mononuclear blood cells were present in large numbers in the tissue cultures of spleen. These the amoebae took in, carried them about for a while, and then gave them off. Several of them would often remain sticking to the posterior end of the amoeba along with many pigment granules. All this weight did not seem to retard the movement of the amoeba.

These results are somewhat similar to those obtained by Smith ('21) with tissue-culture cells, except that the tissue cells take in the melanin pigment at all parts and the pigment granule must always be horizontal to the surface of the cell where it is entering. He notes the formation of vacuoles, but could not determine the ultimate fate of the granules, as the life of the tissue-culture cells is short, and they died without discharging or digesting the granules.

DEATH

Ordinarily when the nucleus and cytoplasm of a tissue-culture cell have taken methylene-blue or brilliant cresyl-blue or neutral-red stain, the cell is said to be dead. Again in tissue-culture cells, when the color of the neutral-red granules fades and they become clear, the cell is thought to be dead (Lewis, '19). With the amoeba a different standard must be used. In one experiment already cited the amoebae were treated with neutral red and with janus black no. 2 at 9:30 in the morning. The neutral red color had disappeared at 12:00, noon, but at 4:30 in the afternoon the amoebae were still moving actively. The next day they were dead.

This is also true of both brilliant cresyl blue and methylene blue. The amoeba moves rapidly after its nucleus and cytoplasm have both become stained. It is evident, therefore, that these old criteria of the death of a cell cannot be applied to an amoeba which still continues to move. Indeed, one can wonder whether the tissue-culture cells are really dead when their nuclei and cytoplasm have taken these stains.

When these amoebae are alive they are actively sending out pseudopodia. Usually these come from only one part of the cell at a time, though any part may send them out. The amoebae not only send out pseudopodia, but they move rapidly. As the animals begin to die, progression is less rapid, though the pseudopodia are formed very quickly. Then gradually the pseudopodia are formed more slowly, then only at one end of the amoeba, and eventually they are simply blebs which flow from one side of the amoeba to the other until finally all motion stops and the amoeba is dead.

From the fact that locomotion and motion become gradually less and less as the amoeba is dying, it seems evident that the motion of an amoeba and not its reaction to vital stains should be the ultimate criterion of life in the trophozoite stage.

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A STATISTICAL NOTE ON EPIDEMIC ENCEPHALITIS *

By RAYMOND PEARL

School of Hygiene and Public Health, The Johns Hopkins University

During the last years of the war and since there has occurred [221] the significant beginning and menacingly rapid spread of a disease either previously not existing, or at least not occurring frequently enough to get general recognition, namely, *encephalitis lethargica* or epidemic encephalitis. Because of its extremely rapid development in frequency of occurrence and because of its extremely fatal character, together with the probability that it may become an epidemic disease of an importance similar to that of poliomyelitis it seems desirable, thus early in its history, to make some analysis of its statistical characteristics. To do this has been the object of the preparation of this paper, in which I have been assisted on the arithmetic side by Mr. John Rice Miner.

The aid which the mathematical analysis of statistical data can render the epidemiologist and pathologist, and the degree of certainty which such analysis can add to their conclusions, are not generally recognized. Usually the health officer is content with the mere tabulation of frequency of occurrence of morbidity and mortality. Such figures are too frequently made the basis of conclusions without any recognition of the errors of sampling involved. Before one can be in any degree certain of conclusions from such figures their probable errors must be known.

Material.—Two papers have recently appeared giving raw data regarding the development of epidemic encephalitis. The

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, The Johns Hopkins University, No. 36.

[221] first of these papers[1] deals with the cases of the disease in the whole United States in the years 1918 and 1919. The second paper[2] deals with the much more restricted field of New York City in 1919 and 1920, but because of the accelerated frequency of occurrence of the disease during the latter year, contains far more statistical material than Smith's paper. It is with the data from this second paper that we shall chiefly deal here.

Case Fatality Rate.—Table 1, taken from (2), gives the incidence and deaths from this disease in New York City in 1919 and 1920. There is a slight correction of an obvious arithmetic error in the total deaths in 1919, from that given in the original.

These data give the following case fatality rates:

$$1919,\ \text{case fatality rate} = \frac{33 \times 100}{128} = 26 \text{ per cent}$$

$$1920,\ \text{case fatality rate} = \frac{211 \times 100}{565} = 37 \text{ per cent}$$

The first of these figures agrees well enough with the 29 per cent given by Smith for the case fatality rate over the whole country in the years 1918 and 1919. The significant thing is that the case fatality rate appears to be increasing. Such a conclusion depends upon the assumption that morbidity from this cause was at least as well reported in 1920 as in [222] 1919. It may be accepted with reasonable certainty that the *deaths* were as well reported in the one year as the other. The well-known high standards maintained in the work of the Bureau of Vital Statistics of the New York City Department of Health practically ensures this. The morbidity figures are more doubtful, because this disease has only recently been made reportable in the city. But let us carefully examine the alternatives which are presented. If we assume (a) that the deaths have been equally well reported in 1919 and 1920, which is a justifiable assumption, and (b) that the true, but unknown, case-fatality rate has remained absolutely constant at the 1919 figure during these two years, then it follows that to get the 37 per cent observed, it will be necessary to suppose that the 565 cases reported in 1920 far *underestimate* the true number which occurred. It must be assumed that actually 812 cases occurred instead of the 565 reported. Now it would seem altogether unlikely, indeed indefinitely improbable, that in the year 1920 there occurred in New York City 247 cases of encephalitis lethargica about which the Department of Health did not know. The *prima facie* reason why this is so improbable is the ever-increasing interest and recognition of the importance [222]

TABLE 1.—INCIDENCE AND DEATHS FROM EPIDEMIC ENCEPHALITIS, BY MONTHS, 1919-1920

Month	1919		1920	
	Cases	Deaths	Cases	Deaths
January	5	..	36	12
February	16	..	149	50
March	25	9	116	52
April	10	1	66	16
May	16	5	42	22
June	4	1	20	12
July	2	1	39	9
August	1	..	28	11
September	8	5	22	6
October	23	7	11	3
November	9	3	12	9
December	9	1	24	9
Totals	128	33	565	211

of this disease by the Department of Health during the period named.

Suppose we consider the second alternative which is that the figures for 1919 greatly underestimate the true and assumed constant case fatality rate. This means, if we assume the deaths to have been substantially accurately reported for the same reason as before, that there were more *cases* reported in 1919 than actually occurred. To justify this second alterna-

[222] tive it must be supposed that actually there occurred in 1919 in New York City only 89 cases of epidemic encephalitis, instead of the 128 reported. This would be a highly improbable assumption. And further we must reckon with the close agreement between Smith's figures of 29 per cent for the case fatality rate for the whole country and the New York 1919 figures of 26 per cent.

Altogether it may be tentatively concluded, with a high degree of probability, that the case fatality rate from the disease is on the increase. But the New York numbers are absolutely small in a statistical sense, and the further question must be asked, as to whether the 11 per cent increase in the case fatality rate between 1919 and 1920 in New York is statistically significant, *i. e.,* greater than might reasonably be supposed to have arisen from chance fluctuation alone.

The problem may be stated in this way: If in a sample of 128 cases of the disease 33 deaths occur, what would be the number of deaths expected in a sample of 565 cases, if nothing but chance were involved in the matter, or in other words if there had been no change in the true case-fatality rate?

We have, by well understood principles (cf. 4 and 5):

Mean deaths expected in second sample of $565 = 148 \pm 16$.

Or, in other words, it is an even chance, if chance alone caused in 1920 fluctuations from the case-fatality rate observed in 1919, that from 132 to 164 deaths would have occurred in 1920, rather than a number smaller than 132 or larger than 164. Actually 211 deaths occurred in 1920. The difference between 211 and 148 is more than 3.9 times the probable error of the latter figure. Whence we may conclude that there was a statistically significant rise in the case fatality rate in 1920 as compared with 1919 in New York City.

Seasonal Distribution.—A question which at once occurs to one in examining Table 1 is this: Is the seasonal distribution (by months) of cases changing as the disease grows in importance, or is the monthly distribution in 1920 different from that of 1919 only by as much as might reasonably be expected from chance sampling?

To determine this question it is necessary only to determine the probability that the two distributions differ signi-

ficantly from each other. It has been shown by Pearson[5, 6, 7] [222]
that such a probability will be given by the expression

$$P=\frac{\int_{\chi}^{\infty}\chi^{q-2}e^{-\frac{1}{2}\chi^2}d\chi}{\int_{0}^{+\infty}\chi^{q-2}e^{-\frac{1}{2}\chi^2}d\chi}.$$

Where

$$\chi^2=NN'S\quad\frac{(f/N-f'/N')^2}{f+f'}\Big]$$

the summation S extending over all pairs of the elemental frequencies f and f'.

From the data of Table 1, we get for the probability that the 1920 and 1919 distribution are two random samples from the same unchanged phenomenal universe

$$\chi^2=89.542,$$

whence it is easily determined that

$$P<.000{,}000{,}1.$$

Or, in words, it appears that less often than once in 10 [223]
million trials would one expect to get true samples of the size here dealt with, as divergent as are the 1919 and 1920 monthly distributions, from the operation of chance alone. Hence we conclude that there was a significant difference in the seasonal incidence of the disease, as indicated by the monthly distribution of cases, in 1919 as compared with 1920 in New York. Examining the details of the χ^2 calculations it is seen that the significant divergences are in the autumn and the spring. There was a significant flare-up in the spring of 1920 lacking in 1919, and a significant autumn outbreak in 1919 lacking in 1920.

Time Lag of Deaths.—On the basis of the 24 months' experience exhibited in Table 1 it is possible to determine the average time that deaths lag behind cases. Put in another way this means the mean duration of *fatal cases* of encephalitis lethargica, between recognized onset and death. This is found to be:

Mean duration of time
between onset and death = 1.046 ± .230 months.

[223] The epidemiological significance of this datum is that it may be expected, in so far as the 1919 and 1920 experience of New York City may be regarded as typical, that the peak of mortality in an epidemic outbreak of this disease will probably occur from 23 to 37 days after the peak of morbidity has been passed. The contrast of this with the time lag in epidemic influenza is sufficiently striking.

Influence of Sex.—Table 2, again taken from (2), gives the sex and age distribution of the cases and deaths in New York City in 1920.

From these data the first question to which attention may be directed is this: Is the difference between the sexes in respect of (a) incidence; or (b) fatality, greater than might reasonably be expected to arise from chance alone, in a city having the sex distribution of its population that New York has?

By elementary principles of probability it is easily found that, among those persons having the disease in New York in 1920, the probability that a given person picked at random would be a male is given by

$$p=\frac{316}{549}=.5756,$$

whence

$$\frac{pq}{n}=.000445.$$

In the whole population of New York (on the basis of the 1910 census figures) the probability that any person picked at random will be a male is

$$p'=.4994 \text{ and}$$

$$\frac{p'q'}{n'}=.000,000,001$$

whence

$$p-p'=.0762\pm.0142$$

and

$$\text{Diff./P.E. Diff.}=5.4.$$

Or, it appears that there is a significantly larger proportion of males among those attacked by epidemic encephalitis than there is among the general poulation. A deviation as large as that between p and p' would occur on the basis of chance alone only once in about every 10,000 trials.

TABLE 2.—ENCEPHALITIS CASES AND DEATHS, BY AGE [223] AND SEX, 1920

Ages	Cases			Deaths		
	Male	Female	Total	Male	Female	Total
Under 5 years....	23	24	47	10	8	18
5– 9 years.......	26	14	40	10	2	12
10–14 "	32	18	50	6	5	11
15–19 "	23	24	47	5	5	10
20–24 "	37	34	71	13	11	24
25–29 "	29	27	56	11	14	25
30–34 "	35	18	53	8	8	16
35–39 "	30	18	48	14	11	25
40–44 "	19	14	33	7	10	17
45–49 "	24	16	40	13	7	20
50–54 "	15	8	23	8	3	11
55–59 "	8	3	11	6	1	7
60–64 "	7	7	14	3	5	8
65–69 "	4	4	8	2	1	3
70–74 "	3	2	5	2	1	3
75–79 "	0	1	1	0	1	1
80–84 "	1	1	2	0	0	0
85 years and over.	..	..	..	..	..	..
Totals..........	316	233	549	118	93	211

Among those *dying* from epidemic encephalitis in New York City in 1920 the probability that any individual picked at random would be a male was

$$p=\frac{118}{211}=.5592.$$

[223] Whence

$$\frac{pq}{n} = .001168$$

$$p' = .4994 \text{ as before.}$$

$$p - p' = .0598 \pm .0230.$$

From this result we may conclude that the proportion of males dying from epidemic encephalitis in New York in 1920 was not significantly different from that found in the general population.

It thus appears that more males were attacked but no more died from encephalitis than would have happened if sex had no differential effect whatever relative to this disease. This [224] result can only mean that the case fatality rate is different in the two sexes.

Influence of Age.—It is stated in (2) that "No age seems exempt, but the greatest proportion of cases occurred in young adults, with a preponderance of males." This raises the following question: Is the age distribution of each of (a) male cases, (b) female cases, (c) male deaths, and (d) female deaths, significantly different, as a whole, from (e) the male population age distribution and (f) the female population age distribution?

Using the χ^2 method, and taking the 1910 age distribution of the New York City population as the basis of comparison, one gets:

For male *cases:*

$$\chi^2 = 8.254.$$

$$P = .410.$$

Or, one would expect in 4 out of every 10 trials with samples of the size here dealt with to get a divergence as great as or greater than that between the male case incidence age distribution and the general population age distribution, if epidemic encephalitis were perfectly random in its age incidence. In other words, there is slight basis for assuming that this disease attacks males more frequently at one age than another.

If we leave out the "under 5" group

$$\chi^2 = 4.111.$$

$$P = .767.$$

This means that in males 5 years old and over the age [224] incidence of cases is very close indeed to a random one. In 77 out of every 100 trials there would result from chance alone deviations as great as or greater than that actually shown between male cases and the general male population.

Turning to male *deaths* we have:

$$\chi^2 = 18.450.$$
$$P = .018.$$

These values indicate a significant divergence of the male death age distribution from the general population male age distribution. But a further analysis gives:

For male deaths under 45 years of age:

$$\chi^2 = 5.790.$$
$$P = .448.$$

For male deaths under 35 years of age:

$$\chi^2 = 3.319.$$
$$P = .652.$$

It thus appears that it is chiefly the excess of male deaths beyond age 45 from this disease that causes the low value of P for the entire male death distribution.

Putting all the facts together it can be asserted, on the basis of the experience of New York City in 1920 with epidemic encephalitis, that in males the disease is probably not significantly more likely to attack one age than another, but instead falls upon males in about the proportion that they are normally present in the general population. It is furthermore not more fatal among the attacked at one age than another until after age 35 is passed. From that point on there are more fatal cases than would be expected if the toll of death were taken purely at random in respect of age.

Turning to the age distribution of female *cases,* and again comparing with the age distribution of the total female population (as of 1910) we get:

$$\chi^2 = 4.847.$$
$$P = .773.$$

It is thus quite certain that, so far as the experience of New York City in 1920 may be taken as typical, epidemic

[224] encephalitis occurs among females without definite age preference, striking the different ages in due proportion as they are represented in the general population. Females of any particular age are not more likely to contract the disease than females of any other age.

The age distribution of *deaths* among females presents a different case. It will be well to examine the facts in some detail. Table 3 gives the data.

TABLE 3.—DATA FOR TESTING THE DEVIATION OF THE FEMALE DEATH AGE DISTRIBUTION FROM THE FEMALE POPULATION AGE DISTRIBUTION

Ages	m Age distribution of female population (as of 1910)	m' Age distribution of females dying of encephalitis lethargica in 1920	$\frac{(m-m')^2}{m}$
Under 5 years......	9.810	8	.3340
5– 9 years........	8.553	2	5.0207
10–14 "	8.268	5	1.2917
15–19 "	9.427	5	2.0790
20–24 "	10.954	11	.0002
25–34 "	17.495	22	1.1600
35–44 "	13.044	21	4.8526
45–64 "	12.554	16	.9459
65 years and over..	2.894	3	.0039
Totals...........	92.999	93	15.6880

$$\chi^2 = 15.6880$$
$$P = .048.$$

It thus appears that the death distribution as a whole is significantly divergent from what would happen if the disease was as likely to be fatal at any given age as at any other. But it is also apparent that more than half of the large value of χ^2 is due to the contribution of but two age groups. These age groups are the $5-9$ and the $35-44$. The deaths in the former were fewer than expected and more than expected in [224] the latter. The numbers are small, however, and it will probably be found, when more data are available, that the facts as to age distribution of deaths are the same in females as in males, namely that there is a definite tendency towards greater fatality in the higher age groups.

SUMMARY [225]

It is distinctly to be understood that the results herein set forth have only such degree of validity as inheres in the experience of New York City with epidemic encephalitis during the two calendar years 1919 and 1920. Doubtless as more material accumulates new statistical relations of this disease will appear. But at the present moment the data here discussed are the most extensive in existence, so far as the writer knows. They fairly represent, in short, about all the basis of a statistical character which exists for forming an intelligent opinion *now* about the statistical features of this potentially important disease. The conclusions reached are, of course, subject to modification by more extensive experience.

What these conclusions are may be summarily stated as follows:

1. In the year 1920 the case incidence of epidemic encephalitis increased in New York City nearly five-fold over 1919. At the same time the case fatality rate increased from 26 per cent of the attacked to 37 per cent of the attacked. It is believed that this increase in case fatality rate is a real phenomenon, and it is certainly statistically significant.

2. The seasonal incidence, as judged by monthly distribution of cases was significantly different in 1920 to what it was in 1919.

3. The peak of mortality in an epidemic outbreak may be expected to occur from 23 to 37 days *after* the peak of case incidence (morbidity).

4. There is a significantly larger proportion of males among the attacked than there is in the general population, or, put in another way, males are especially susceptible, to a statistically significant degree.

[[illegible]] 5. Deaths occur among males no more frequently in comparison with females than would be expected from the normal proportions of the two sexes in the population at large.

6. The disease is not more likely to attack either males or females at one age than at another. The age distribution of attacked cases, in other words, does not significantly differ in either sex from the age distribution of the general population.

7. The age distribution of deaths does differ significantly in both sexes from the age distribution of the population. There appears to be a definite tendency for the disease to be more fatal in the higher age groups.

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RAYMOND PEARL

A biological classification of the causes of death (1)

Some time ago the writer (2) proposed a biological classification ie causes of death, as set forth in the International List and by statisticians all over the world. The underlying idea in suggested classification was to put together all those lethal ties which bring about death because of the functional break- n of the same general organ system. In the idea of « break- n » as here used is included both actual destruction of the n in greater or less degree, through lesions; and also cessation unction quite apart from anatomical lesion. The cause of the tional breakdown from the point of view of this classification be anything whatever in the range of pathology. It may lt from bacterial infection, or from trophic disturbances, or damage due to mechanical disturbances which prevent the inuation, of normal function, or to any other cause whatever. ther words, the basis of this classification is not that of patho- cal causation, but it is rather that of functional, organic kdown. We look at the question of death from the standpoint he pure biologist, who concerns himself not with what causes cessation of function, but rather with what part of the orga- n ceases to function and therefore causes death.

It was emphasized in the first publication on this subject, and ould repeat that emphasis here, that this new classification is advocated for general statistical purposes. As purpose is purely lytical, for the further study of certain problems of biology erlying human mortality statistics. These statistics are now uniformly, and, on the whole, fairly satisfactorily, tabulated under the rubrics of the International List. Uniformity in statistical classification is essential to useable practical vital statistics. Such uniformity has now become well established through the International Classification. It would be most undesirable, and the writer would strongly oppose, making any radical changes in the International Classification.

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(1) Papers from the Department of Biometry and Vital Statistics, ol of Hygiene and Public Health, Johns Hopkins University, N. 19.

(2) PEARL, R. *Certain Evolutionary Aspects of Human mortality es. American Naturalist*, Vol. LIV. pp. 5-44. 1920.

— *On the Embryological Basis of Human Mortality. Proc. Nat. Acad*, Vol. 5, N.° 12, pp. 593-598, 1919.

Cfr. also *Mortality Classified from a New Standpoint.* Editorial. *rn. Amer. Med. Assoc.* Vol. 74, N.° 23, June, 5, 1920, pp. 1579 and 1580.

The classification presented here has attracted some attention as a result of its publication in a purely biological journal, and it seems desirable to set forth in useable form the details of the classification in a publication more readily accessible to vital statisticians than that in which the first discussion of the subject appeared. There is the more reason for doing this from the fact that there will shortly appear from the writer's laboratory several detailed statistical studies based upon this classification.

In order to save space no attempt will be made here to repeat the pathological discussions relative to the inclusion of specific causes of death in the groups where they are placed in classification. Anyone wishing to examine at first hand the reasoning which led to the details of the makeup of this classification must consult the original papers referred to above. It should be said that in respect of a number of details the classification is still to be regarded as tentative. The writer will welcome suggestions as to modifications calculated to bring its details more in accord with the fundamental idea on which the classification as a whole is based.

The details of the classification may be set forth in two ways, which together serve as a cross index to all of the 189 causes of death in the International List. The biological classification proposes ten main groups as follows:

Main Heads of Biological Classification of Causes of Human Mortality.

All human mortality is due to the breakdown and failure to function of one or another of the bracketed organ groups

I. Circulatory system, blood, and blood-forming organs.
II. Respiratory system.
III. Primary and secondary sex organs.
IV. Kidneys and related extretory organs.
V. Skeletal and muscular systems.
VI. Alimentary tract and associated organs concerned in metabolism.
VII. Nervous system and sense organs.
VIII. Skin.
IX. Endocrinal system.

or to X. All other causes of death.

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X. *All other causes of death.*

Included causes: 10, 34, 45, 46, 55, 148, 152 (other caus peculiar to early infancy only), 153, 154, 164-186 incl. (all exter and accidental causes except suicide), 187-189 incl. (ill-define diseases, total).

It will be noted that two causes of death (viz. 21, Glander and 21, Anthrax) have been transferred from Class VIII (the ski where they were placed in earlier papers, to Class I (the circulatory system, blood, and blood-forming organs). Further consideration of the matter since the first study leads to the conclusion that these diseases should go from a biological point of view in the same class with purulent infection and septicemia. For the detailed arguments on the point here at issue my first paper on the subject should be consulted. It is to be noted that, because of the insignificant death rate from causes 21 and 22 no essential differen in any statistical conclusions will be made wherever these two diseases are placed.

In order to facilitate the finding of the main group in which a particular cause of death of the International List falls the following index will be found useful:

Index to Biological Classification of Causes of Death.

International N.°	Cause	Biological N.°
1	Typhoid fever.	VI
2	Typhus fever	I
3	Relapsing fever	I
4	Malaria	I
5	Smallpox	VIII
6	Measles	II
7	Scarlet fever	I
8	Whooping cough.	II
9	Diphtheria and croup	II
10	Influenza.	II
11	Miliary fever	I
12	Asiatic cholera	VI
13	Cholera nostras	VI
14	Dysentery	VI
15	Plague	I
16	Yellow fever	I
17	Leprosy	VII
18	Erysipelas	VIII

onal	Cause	Biological N.°
	Other epidemic diseases	X
	Purulent infection and septicemia. . . .	I
	Glanders.	I
	Anthrax	I
	Rabies	VII
	Tetanus	VII
	Mycoses	VIII
	Pellagra	VI
	Beriberi	VI
	Tuberculosis of the lungs.	II
	Acute miliary tuberculosis	II
	Tuberculous meningitis	VII
	Abdominal tubercolosis	VI
	Pott's disease	V
	White swellings	V
	Tubercolosis of other organs	X
	Disseminated tuberculosis.	VI
	Rickets	V
	Syphilis	III
	Gonococcus infection	III
	Cancer of the buccal cavity.	VI
	Cancer of the stomach, liver	VI
	Cancer of the peritoneum, intestines, rectum	VI
	Cancer of the female genital organs. . .	III
	Cancer of the breast	III
	Cancer of the skin	VIII
	Cancer of other organs or of organs not specified	X
	Other tumors (tumors of the female genital organs excepted)	X
	Acute articular rheumatism	V
	Chronic rheumatism and gout	V
	Scurvy	VI
	Diabetes	VI
	Exophthalmic goiter.	IX
	Addison's disease	IX
	Leukemia	I
	Anemia, chlorosis	I
	Other general diseases.	X
	Alcoholism (acute or chronic)	VII
	Chronic lead poisoning.	VI
	Other chronic occupation poisonings . . .	VI
	Other chronic poisonings	VI

X. *All other causes of death.*

Included causes: 19, 34, 45, 46, 55, 148, 152 (other causes peculiar to early infancy only), 153, 154, 164-186 incl. (all extern and accidental causes except suicide), 187-189 incl. (ill-defin diseases, total).

It will be noted that two causes of death (viz. 21, Glanders and 21, Anthrax) have been transferred from Class VIII (the skin) where they were placed in earlier papers, to Class I (the circulatory system, blood, and blood-forming organs). Further consideration of the matter since the first study leads to the conclusion that these diseases should go from a biological point of view in the same class with purulent infection and septicemia. For the detailed arguments on the point here at issue my first paper on the subject should be consulted. It is to be noted that, because of the insignificant death rate from causes 21 and 22 no essential difference in any statistical conclusions will be made wherever these two diseases are placed.

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6	Measles	II
7	Scarlet fever	I
8	Whooping cough	II
9	Diphtheria and croup	II
10	Influenza	II
11	Miliary fever	I
12	Asiatic cholera	VI
13	Cholera nostras	VI
14	Dysentery	VI
15	Plague	I
16	Yellow fever	I
17	Leprosy	VII
18	Erysipelas	VIII

onal	Cause	Biological N.°
	Other epidemic diseases	X
	Purulent infection and septicemia. . . .	I
	Glanders.	I
	Anthrax	I
	Rabies	VII
	Tetanus	VII
	Mycoses	VIII
	Pellagra	VI
	Beriberi	VI
	Tuberculosis of the lungs.	II
	Acute miliary tuberculosis	II
	Tuberculous meningitis	VII
	Abdominal tubercolosis	VI
	Pott's disease	V
	White swellings	V
	Tubercolosis of other organs	X
	Disseminated tuberculosis.	VI
	Rickets	V
	Syphilis	III
	Gonococcus infection	III
	Cancer of the buccal cavity.	VI
	Cancer of the stomach, liver	VI
	Cancer of the peritoneum, intestines, rectum	VI
	Cancer of the female genital organs. . .	III
	Cancer of the breast	III
	Cancer of the skin	VIII
	Cancer of other organs or of organs not specified	X
	Other tumors (tumors of the female genital organs excepted)	X
	Acute articular rheumatism.	V
	Chronic rheumatism and gout	V
	Scurvy	VI
	Diabetes	VI
	Exophthalmic goiter.	IX
	Addison's disease	IX
	Leukemia	I
	Anemia, chlorosis	I
	Other general diseases.	X
	Alcoholism (acute or chronic)	VII
	Chronic lead poisoning.	VI
	Other chronic occupation poisonings . . .	VI
	Other chronic poisonings	VI

International N.°	Cause	Biologic N.°
137	Puerperal septicemia	III
138	Puerperal albuminuria and convulsions	IV
139	Puerperal phlegmasia alba dolens, embolus, sudden death	III
140	Following childbirth (not otherwise defined)	III
141	Puerperal diseases of the breast	III
142	Gangrene	I
143	Furuncle	VIII
144	Acute abscess	VIII
145	Other diseases of the skin and annexa	VIII
146	Diseases of the bones (tuberculosis excepted)	V
147	Diseases of the joints (tuberculosis and rheumatism excepted)	V
148	Amputations	X
149	Other diseases of the organs of locomotion	V
150	Hydrocephalus	VII
150	Congenital malformations of the heart	I
150	Other congenital malformations	VI
151	Congenital debility: premature birth	III
151	Congenital debility: atrophy, marasmus, etc.	VI
152	Injuries at birth	III
152	Other causes peculiar to early infancy	X
153	Lack of care	X
154	Senility	X
155 to 163 incl.	Suicide (total)	VII
164 to 186 incl.	All external and accidental causes except suicide	X
187 to 189 incl.	Ill-defined diseases (total)	X

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BIOMETRIC DATA ON INFANT MORTALITY IN THE UNITED STATES BIRTH REGISTRATION AREA, 1915–1918.*

By RAYMOND PEARL.

(Received for publication May 20th, 1921.)

Introduction.

Until recently it has been impossible to discuss on a satisfactory basis the infant mortality of any considerable portion of the United States. This difficulty has arisen from the fact that except in a few localities, notably some of the New England States, there has been in the past no adequate system of birth registration. The most accurate practical method of stating the force of infant mortality is to relate the number of deaths of infants under one year of age in a given time unit to the number born in the same time unit. The expression of infant mortality rates as deaths under one year per thousand births in the same time interval is not entirely accurate in theory. Instead of using the "same time interval" one should use the births of an "equivalent" year to get results of the highest accuracy, as has been pointed out by Knibs (1917). But to a first approximation the ordinary method of using the same time interval for both the births and the deaths is sufficiently accurate.

One must have accurate birth statistics before infant mortality can be adequately discussed. Theoretically, of course, one might use in census years the count of the population of infants under one year of age as the denominator of the mortality rate fraction or the estimated population for the same age group in inter-censal years. Such procedure has, however, never been regarded by vital statisticians as entirely satisfactory because of the large errors which are known to inhere in counts of the infant population. Recently Crum (1920) has, apparently on the somewhat dubious ground of immediate convenience and expediency, used for the study of infant mortality, an "infant mortality index" which is defined as "the infant mortality per 1000 of estimated population under one year of age." Regarding this index he says:

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* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 39. A brief preliminary report of this work has been published elsewhere (cf., Pearl, R., 1920 and 1921).

"Infant mortality rate has come to mean infant deaths per 1000 births, the deaths and births being for the same period—week, month or year, as the case may be. For comparative purposes the 'index' as here determined is probably as accurate, if not more so, that the 'rate' as births are nowhere so completely reported as deaths and the variation in the completeness of birth reporting is considerable, even in the large American cities."

This would seem to be distinctly a retrograde step in biostatistical research. Furthermore one cannot agree that this index is "probably as accurate, if not more so," than the usual rate, particularly in view of the well known inaccuracy of even total population estimates towards the ends of inter-censal periods, which is just the point at which Crum is working. We may well expect, in view of the fact that the general United States population curve has passed its point of inflection (cf. Pearl and Reed, 1920) that for some time population estimated by the usual methods will be particularly inaccurate. Again, in such a case as Crum's usage, the estimate is not of the total population, but of one small age group. Also during the war period industrial movements connected with the war profoundly disturbed the normal population growth of large cities. Altogether it is believed that the safest ground is to stick to the normal infant mortality rate per 1000 living births.

It is a matter of great satisfaction that at last there is well established a Birth Registration Area for the United States, and five annual reports on Birth Statistics of this area have been issued to date by the Census Bureau. We are well embarked now on the policy of adequate birth statistics for the country, and unquestionably within a comparatively few years the Birth Registration Area will cover the major portion of the country as the Death Registration Area now does. In the short period since the Birth Registration Area has been established its growth in extent has been gratifyingly rapid. The first report on birth statistics for the year 1915 comprised data from an area including approximately only 31 per cent. of the population of the country. The 1918 birth statistics report gives data from an area including 53 per cent. of the population. This furnishes a sufficient volume of material so that one may begin the mathematical analysis of some of the problems of infant mortality with some assurance of reaching valid conclusions.

The purpose of the present paper is primarily to present the facts, with some simple mathematical analysis, of *variation* in rate of infant mortality in different geographic or demographic units of the population. These facts of variation make it clearer where the fundamental administrative problems of control of infant mortality lie than perhaps could be done in any other way. The first step in the solution of any problem is obviously a clear definition of the problem itself. We shall see, as we pass from city to city, town to town, or rural country to rural country, that the rate of infant mortality varies greatly. In the hypothetical community where the most perfect administrative control over infant mortality possible or conceivable had been attained this variation would to a considerable extent disappear, the only residue of diversity between communities in respect of infant mortality being such as arose either (1) purely by the operation of chance, that is, from random sampling, and (2) from the racial composition of the several populations, and (3) from fundamentally uncontrollable environmental differences, such as climate, soil, etc. Now with the actually existing condition of variation between different communities in respect of infant mortality, it is obvious that there must be particulate and presumably in large degree determinable reasons for each particular difference which exists. Equally obviously, before administrative control can effectively wipe out these mortality differences and get all communities at or near the level of the lowest, we must know something about the determining causes upon which they depend. Operating on a basis largely of empiricism and *a priori* reasoning, efforts to reduce infant mortality have in the past been attempted with considerable success. Also, with the advance of general sanitation the death-rate under one year of age has fallen enormously. Greenwood (1912) quotes some interesting figures on the point from Farr, which we may well reproduce here to show how enormous has been the improvement.

TABLE 1.

Showing the reduction in the mortality of infancy and early childhood. (After Greenwood.)

Period.	1730–49.	1750–69.	1770–89.	1790–1809.	1810–29.
Percentage deaths under 5 years	74.5	63.0	51.5	41.3	31.8

But after such a decline as these figures indicate, to continue the reduction presents a difficult problem to the administrative official. The easy part of the conflict has happened and is in the past. To continue the good fight with the same relative measure of success, one presently must needs know more precisely than is now known the pattern of the causal nexus which controls and determines the rate of infant mortality. The problem confronts the administrative official or the altruistic organization in a specific rather than a general manner. City A has a death rate under one year of age so low that even the most sanguine of hygienic optimists would hardly undertake seriously to reduce it further by any significant amount. In City B, on the other hand, babies die like flies, only somewhat more rapidly. City B differs in many respects from A. Some of these respects are such as to be easily within the power of control of a health official. Others, such as climate or the racial composition of the population, for example, are obviously beyond the possibility of any control or modification. Others lie between the two extremes, and offer practical difficulties of varying degrees. What one needs to know is which particular line of effort will in practice yield the largest return. And it is real knowledge not *a priori* logic that is wanted. Let a single example illustrate. It has been maintained that excessive infant mortality is primarily the resultant of the so-called "degrading influence" of poverty, and such a contention stirs a warmly sentimental feeling of agreement in the minds of a well-meaning public, zealous to do good. This relationship obviously *ought* to be true, therefore to a too-common type of mind it must be and is true. But Greenwood and Brown (1912) in what may fairly be regarded the most thoroughly sound, critical and penetrating contribution which has yet been made to the problem of infant mortality, are unable "to demonstrate any unambiguous association between poverty . . . and the death rate of infants."

The plain fact is that before control or ameliorative measures can be applied with the maximum of efficient economy to the general public health problem of infant mortality we must know a great deal more than we now do about the quantitative influence of the general factors which induce spatial and temporal differences in the rate of that mortality. But first we must get an adequate conception of the magnitude and character of the differences themselves. Let us, therefore, turn to the examination of the facts regarding variation in infant morality in the United States Birth Registration Area.

VARIATION DATA.

The material used in the following discussion has been extracted chiefly from the four annual reports on Birth Statistics issued to date. In Table 2 is given the frequency distribution showing variation in the rate of infant mortality (deaths per thousand living births) for the following groups:

1. Total population in cities of population of 25,000 or over in 1910.
2. Total population in cities of under 25,000 population in 1910.
3. Total population in rural counties of registration states.
4. White population in cities of population of 25,000 or over in 1910.
5. White population in cities of under 25,000 population.
6. White population in rural counties of registration states.
7. Colored population in cities of population of 25,000 or over in 1910.
8. Colored population in cities of under 25,000 population.
9. Colored population in rural counties of registration states.

The entries in the table are frequencies, and are to be read in accordance with the following example. There were in 1915, 29 cities of 25,000 population or more (in 1910), in the Birth Registration Area, having an infant mortality rate falling somewhere between 100 and 119 deaths under one year of age per thousand living births. All other entries are to be read in the same manner.

In order to make possible a better appreciation of the nature of the frequency distributions exhibited in Table 2, Figs. 1 and 2 have been prepared. These show for the year 1918 the frequency polygons for the total population of (*a*) cities of 25,000 and over, (*b*) cities of under 25,000, and (*c*) rural counties, and also the distributions for the white and colored population of rural counties in 1918.
and space need not be taken for the graphic presentation of the others. From Table 2 and Figs. 1 and 2 the following points are to be noted:

1. The most striking immediately observable feature of these distributions is the great range of variation which they exhibit. For example, in 1918 the 236 cities of under 25,000 inhabitants showed infant mortality rates ranging all the way from the class 40–59 deaths per thousand births to the class 300–319 deaths per thousand births. The range of variation is even greater than this in the case of the distributions for the colored population. Thus the 27 cities of 25,000 and over for which the population was divided in respect of color in 1918 show a range of variation of infant mortality from the class

TABLE 2.

*Frequency distribution showing variation in **infant mortality in Birth Registration** Area of the United States.*

Deaths per 1000 births in specified years.	Total population cities of 25,000 and over.*				Total population cities of under 25,000.*				Total population rural counties.				White population cities of 25,000 and over.*		White population cities under 25,000.*		White population rural counties.		Colored population cities of 25,000 and over.*		Colored population cities under 25,000.*		Colored population rural counties.	
	1915	1916	1917	1918	1915	1916	1917	1918	1915	1916	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918	1917	1918
0– 19	—	—	—	—	1	1	—	—	1	—	3	1	—	—	—	—	—	—	—	—	—	—	—	1
20– 39	—	1	—	—	—	2	1	—	2	9	33	32	—	—	—	—	1	9	—	—	—	—	2	1
40– 59	2	1	5	1	11	4	12	6	49	45	152	174	1	—	—	1	30	33	—	—	—	1	8	5
60– 79	16	18	22	17	25	27	49	37	130	125	396	342	4	1	6	3	71	63	—	1	1	—	26	22
80– 99	27	24	50	43	44	42	76	65	99	107	316	298	14	13	8	5	66	64	—	1	1	1	29	19
100–119	29	34	45	40	35	39	61	48	52	57	140	165	7	8	8	6	46	38	—	1	3	1	36	42
120–139	13	14	13	27	20	23	24	38	17	21	59	64	1	5	3	4	13	16	3	1	—	4	40	37
140–159	9	5	7	13	11	7	13	15	6	15	18	31	—	—	1	5	3	8	1	2	3	1	28	30
160–179	1	2	1	1	3	5	5	15	1	2	4	11	—	—	—	2	1	2	6	2	1	2	21	19
180–199	1	—	1	2	3	6	4	4	—	—	4	8	—	—	—	—	1	—	5	2	—	—	18	17
200–219	—	—	—	—	—	—	—	6	1	—	1	1	—	—	—	—	—	1	6	5	2	2	5	10
220–239	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	3	6	5	4	8	12
240–259	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	2	2	3	1	4	8
260–279	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	1	4
280–299	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	4	2	2
300–319	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	1	2	—	1
320–339	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	—
340–359	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—
360–379	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
380–399	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
400–419	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
420–439	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—
440–459	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
460–479	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
480–499	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
500–519	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—
520–539	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
540–559	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
560–579	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
580–599	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
600–619	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
	98	99	144	144	153	156	236	236	358	381	1127	1127	27	27	26	26	232	234	27	27	26	26	232	234

These diagrams are fairly representative of all the distributions 79 deaths under one per thousand births, to the class 500–519 deaths er one per thousand births. The most extreme range is shown by distribution for the colored population of rural counties in 1917,

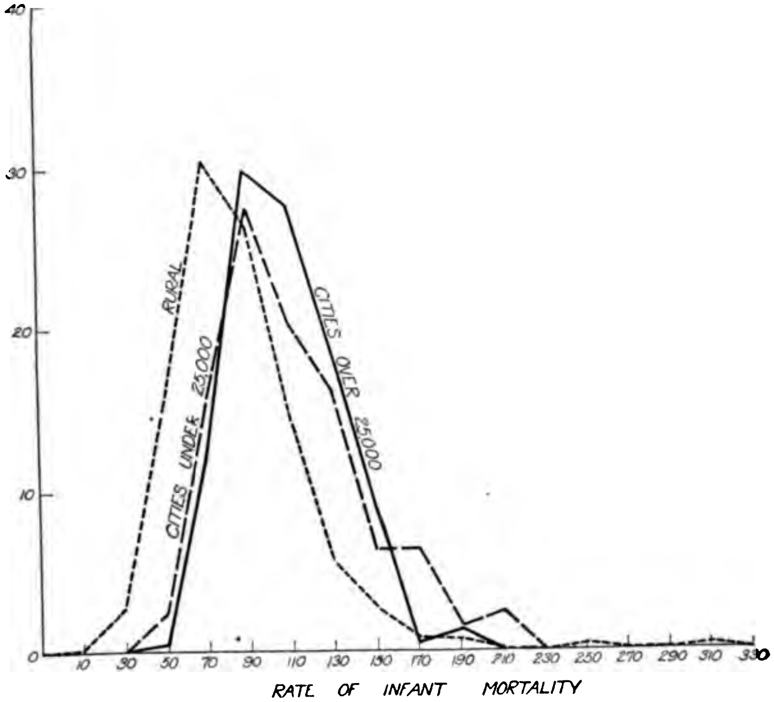

Fig. 1. Frequency polygons showing variation in the rate of infant mortality 918 for the total population of (*a*) cities of 25,000 and over, (*b*) cities of r 25,000, and (*c*) rural counties.

re the distribution extends from class 20–39 deaths under one per sand births to the class 600–619 deaths under one per thousand ns. These extraordinarily large ranges of variation demonstrate aps more clearly than could be done in any other way the opporty which exists for effective administrative control and reduction fant mortality. If there are communities, as there are in plenty, ing infant mortality rates under 100 deaths per thousand births, ggests at once that it is possible, if the right measures are systically and effectively applied, to reduce the infant mortality in e other communities showing very high rates to something like level of these at present more fortunate communities.

2. The distributions are all plainly of the "cocked-hat" type. There is no evidence anywh distributions involving only relatively few commu towards more than a single mode.*

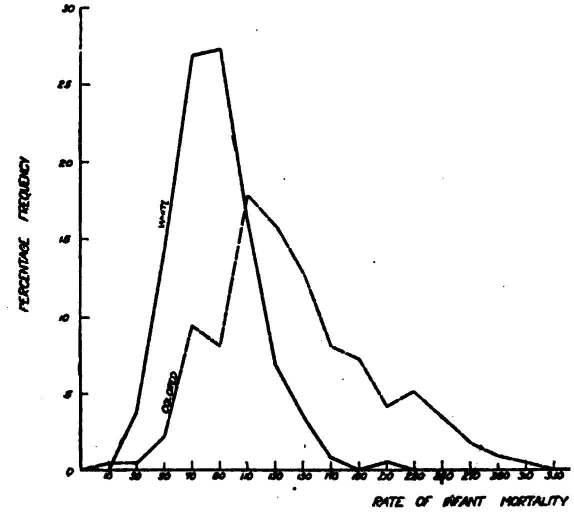

FIG. 2. Frequency polygons showing variations in 1918 of (*a*) the white population, and (*b*) the colored pop

3. These distributions impress anyone who is variation curves as being unusually peaked in t mode. In other words, instead of presenting a r modal region the tops of these curves tend rat We shall see later when discussing the analytic curves that this peaked characteristic evident on out by the mathematical analysis.

In Table 3 are presented the chief physical c tributions of Table 2. These constants have bee method of moments from the original raw data.‡

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* For the benefit of readers not entirely familiar wit culus it may be said that the mode of a frequency curv maximum ordinate of the curve falls. It represents the able" occurrence. Analytically, it is the point on the cu

† For a very brief and summarized introduction to t treatment of frequency curves see Pearson, K. (1914), p to the basic literature on the subject will be found there.

‡ I am greatly indebted to my assistants, Mr. Joh Charmian Howell, for aid in the arithmetical work of this

erable grouping used in making up these distributions, and also because they all exhibit clear evidence of high contact at both ends of the range, Sheppard's corrections for the moments have been used throughout. In the case of the distributions divided in respect of color of the population it has not been thought advisable to calculate the higher analytical constants except in the case of the 1917 and 1918 rural distributions. In the other cases the numbers involved are too small to make the analytical treatment of any particular meaning or value.

The constants tabled are:

1. The arithmetic mean.
2. The median. This measures the value above and below which exactly half of the variates occur.
3. The mode. This is calculated from the relation

$$\text{Mode} = \text{Mean} - d,$$

where,

$$d = \sigma \times sk.$$

Its significance has already been pointed out.

4. The standard deviation denoted by σ. This constant measures in absolute units the degree of "scatter" or variation exhibited by the distribution.
5. The coefficient of variation. Theoretically this constant, which is the percentage of the standard deviation in the mean, has no place in discussions of variation of rates or other forms of percentages. With this fact clearly in mind I have, however, decided to table for these distributions the percentage which the standard deviation is of the mean, chiefly because of the fact that the mean rates exhibited by these distributions differ widely, particularly in the case of white and colored populations, and it seems useful to relate the variation to the mean in these cases.
6. The skewness. This constant measures the degree of asymmetry of a frequency distribution. If a distribution is perfectly symmetrical on both sides of the mean so that if folded over upon the mean as an axis the two limbs would exactly coincide, the value of the skewness is zero. It has been calculated in the present instance from the general formula,

$$sk = \frac{\sqrt{\beta_1}(\beta_2 + 3)}{2(5\beta_2 - 6\beta_1 - 9)}$$

TABLE 3.

Constants of variation in rate of infant mortality (deaths under 1 per 1000 births) computed from data of Table 1.

Group.	Mean.†	Mode.†	Median.†	Standard† deviation.	Coefficient of variation (per cent.).	Skewness.	Kurtosis.
Cities over 25,000,* Total, 1915......	104.49± 1.78	96.26	102.76	26.14±1.26	25.02±1.28	+.3148±.0937	.145± .491
" " " " 1916......	102.53± 1.67	104.47	103.24	24.69±1.18	24.09±1.22	−.0786±.0848	.224± .511
" " " " 1917......	99.58± 1.32	93.83	98.00	23.45± .93	23.55± .99	+.2455±.0858	1.127±1.558
" " " " 1918......	107.78± 1.41	99.66	105.50	25.07±1.00	23.26± .96	+.3237±.0800	.271± .471
Cities under 25,000,* Total, 1915....	100.98± 1.68	92.87	97.95	30.81±1.18	30.51±1.28	+.1934±.0657	.419± .603
" " " " 1916....	104.23± 1.75	97.05	101.03	32.38±1.24	31.07±1.30	+.2217±.0678	.725± .916
" " " " 1917....	99.24± 1.32	84.74	94.74	29.94± .93	30.17±1.02	+.4840±.1197	1.624±1.197
" " " " 1918....	111.61± 1.66	90.36	104.17	37.78±1.17	33.85±1.13	+.5625±.2647	3.212±2.945
Rural counties, Total, 1915.........	83.07± .85	75.40	79.54	23.95± .60	28.83± .79	+.3204±.1454	2.200±2.690
" " " 1916.........	85.28± .90	76.10	82.15	29.94± .63	30.42± .81	+.3536±.0509	.362± .319
" " " 1917.........	82.01± .52	74.73	78.96	25.71± .37	31.35± .49	+.2833±.1157	2.392±2.379
" " " 1918.........	84.43± .57	72.14	80.97	28.40± .40	33.64± .48	+.4328±.0409	1.281± .482
Cities over 25,000,* White, 1917.....	92.22± 2.02	—	92.14	15.60±1.43	16.91±1.60	—	—
" " " " 1918.....	102.59± 2.00	—	99.23	15.42±1.42	15.03±1.41	—	—
Cities under 25,000,* White, 1917....	98.46± 2.75	—	97.50	20.82±1.95	21.15±2.06	—	—
" " " " 1918....	114.62± 4.17	—	113.33	31.49±2.95	27.47±2.80	—	—
Rural counties, White, 1917.........	86.21± 1.07	81.66	84.24	24.15± .76	28.02± .99	+.1799	2.847
" " " 1918.........	85.90± 1.27	77.80	83.75	28.90± .90	33.65±1.05	+.2802±.0650	.999±1.055
Cities over 25,000,* Colored, 1917....	202.59± 8.88	—	194.00	68.43±6.28	33.78±3.44	—	—
" " " " 1918....	216.67±11.15	—	214.00	75.87±7.88	39.63±3.65	—	—
Cities under 25,000,* Colored, 1917 ..	213.08± 9.92	—	228.00	74.96±7.01	35.18±3.68	—	—
" " " " 1918 ..	217.69±11.46	—	225.00	86.65±8.10	39.80±4.27	—	—
Rural counties, Colored, 1917.......	134.76± 2.55	106.17	127.25	57.37±1.80	42.57±1.56	+.4984±.5222	4.036±6.160
" " " 1918.......	147.26± 2.92	108.83	134.59	66.15±2.06	44.92±1.66	+.5819±.4154	4.239±4.998

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* In 1910.

† In concrete units, i.e., rate of deaths under 1 per 1000 births.

7. The kurtosis. This constant which in terms of the moments has the following value

$$\eta = \beta_2 - 3$$

measures the extent to which a curve in the region about the mode is more or less flat-topped than the normal or Gaussian curve. The introduction of this constant we owe to Pearson (1905). Curves on which the kurtosis has a negative value ($\beta_2 < 3$) are more flat-topped than the normal curve and are called platykurtic, while those which are more peaked than the normal curve have a positive value of the kurtosis ($\beta_2 > 3$) and are called leptokurtic.

From the data presented in Table 3 the following points are to be noted:

1. There is no certainly significant decline in the mean value of the rate of infant mortality during the four years covered by these statistics in any of the demographic units considered. In the cities of over 25,000 the mean rate declined during the years 1915, 1916, and 1917, but the total amount of this drop cannot be regarded as statistically significant, having regard to the probable errors involved. In other words, the change from a mean rate of 104 in 1915 to a mean rate of 100 (99.58) in 1917 may easily have been simply the result of chance.

2. In 1918 there was a general tendency towards an increase in the mean rate of mortality over that which obtained in 1917. This increase is unquestionably to be attributed to the influenza epidemic of the autumn and winter of 1918. A careful examination of the rates by months will convince one that the mortality of infants increased very materially during the period of the epidemic. Whether this increased number of deaths was truly to be charged to influenza does not concern us here. The important fact is that the rate of infant mortality markedly increased coincidently with the existence of the epidemic. In a number of cases the increase in the mean rate of 1918 over 1917 is probably statistically significant having regard to the probable errors involved. Thus we have the following large differences:

Cities under 25,000, Total, 1918 mean to 1917 mean $= 12.37 \pm 2.12$
Cities over 25,000, White, 1918 mean to 1917 mean $= 10.37 \pm 2.84$
Cities under 25,000, White, 1918 mean to 1917 mean $= 16.16 \pm 5.00$

It is noteworthy that this increase in the infant mortality rate in 1918 is practically confined entirely to the cities. The rural counties, whether for white or colored or total population, show little or no change in 1918 as compared with 1917.

3. There is no unequivocal difference in the mean rates of infant mortality in the larger as compared with the smaller cities. Considering the largest differences in mean rates for total populations in cities of 25,000 and over, as compared with cities of under 25,000, there is no difference which is as much as even three times its probable error. This result that there is no marked or striking difference in the mean rate of infant mortality in large as compared with small cities is somewhat surprising. It suggests by inference that when the matter is adequately investigated it will probably be found that there is no definite or significant correlation between the rate of infant mortality and the density of population in American cities. It should be understood, however, that this is here suggested only as a probable inference. Positive statements on the matter cannot be made until the point has been carefully investigated by the method of multiple correlation. The suggestion which these figures make, however, is in accord with the results of the writer's studies (Pearl, 1919 and 1921) on the correlation between the influenza mortality and density of population, where it is shown that there is no sensible net correlation between these two variables.

4. The mean rates of infant mortality are notably smaller in the rural than in the urban areas. This fact has, of course, long been well known. The first writer on vital statistics, in the sense in which we now understand that subject, Captain John Graunt (1662), more than 250 years ago pointed out that rural communities exhibited generally a lower rate of mortality than urban communities. We are still nearly as far as he was from a scientific understanding of *why* this is so. There has long been current a certain glib patter of explanation for the superiority of rural communities over urban in rate of mortality, but the subject still awaits careful analytical quantitative investigation, which will measure the relative influence of each one of the considerable number of factors which may be obviously directly concerned in producing this difference. The difference between urban and rural rates of infant mortality is reflected just as clearly in the high absolute rates of the colored population as it is in the lower rates of the white population.

5. The mean rates of infant mortality are, roughly speaking, some-

thing like twice as high for the colored population as for the white population in each of the demographic units considered, and at all times. This, again, is a fact in general well known, but here we have precise figures on the point, with probable errors, which show definitely how tremendously poorer the negro baby's chances of surviving the first year of life are than the white baby's.

6. The mode is seen in every case but one (1916, cities over 25,000, total) to be smaller than the mean; that is, to lie to the left of the mean in the distribution. The differences between mean and mode are fairly considerable for most of the distributions. They are largest in the case of the rural colored distributions.

7. The cities of over 25,000 exhibit distinctly less variation in respect of infant mortality than do either the smaller cities (under 25,000) or the rural counties. This is true, however the variation is measured, whether absolutely, in terms of standard deviation, or relatively in percentage terms. The smaller cities and the rural counties exhibit about the same degree of variation relative to their means, but absolutely, in terms of standard deviation, the rural counties show less variability than the cities under 25,000. It is probable that in this case the coefficient of variation represents more truly the real biological fact than the standard deviation. The colored distributions exhibit a much higher degree of variation in respect of infant mortality, however measured, whether absolute or relative, than do the white populations. Probably part of this greater variation in the colored population arises from the fact that these populations are absolutely much smaller in size in every case than the white populations of the same communities, and therefore less likely to give steady and characteristic rates. How much of the actually observed variation, however, is to be explained in this way is at present undeterminable. In general, it may fairly be assumed that the greater the variation exhibited by a given class of the community in respect of infant mortality, the greater the chance of effective control and reduction of the average infant mortality by administrative measures. There can be no question that there is no field which offers so great opportunities in this direction as the colored population.

8. The skewness is seen to be positive in sign in every case but one. In that case (1916, cities over 25,000, total) the skewness is not significant in comparison with its probable error. With this exception the curves tend to tail off more gradually and farther toward the right end than toward the left end of the range, and in consequence,

as we have already seen, the mode lies to the left of the mean. In many instances (notably in the distributions for the colored population of rural counties) the skewness values rise to considerable magnitudes and may be regarded as significantly different from zero, having regard to their probable errors. In other words, the rate of infant mortality in these different American demographic units tends generally to distribute itself in a substantially unsymmetrical fashion about the mean, extremely high rates occurring more frequently than correspondingly low rates. This fact might perhaps be taken to indicate that the task confronting the administrative control of infant mortality in certain communities of the United States and yet to be accomplished is even greater than what has already been accomplished in the past, great and worthy of commendation as that is.

9. The kurtosis is seen to be positive in sign and relatively large in amount in most cases. This confirms analytically the conclusion already reached from mere inspection, namely, that the curves of variation in infant mortality are, with great uniformity, leptokurtic; that is, more sharply peaked than the corresponding normal curve would be.

10. A noteworthy point is the remarkable similarity, evident both from inspection and from the analytical constants, in all of these frequency distributions. Evidently infant mortality variation curves are of a quite definitely characteristic and uniform type, at least in this country. It will be interesting to examine similar curves for other countries.

For the sake of further analytical work the moments and certain derived constants from the longer distributions are given in Table 4.

It is of some interest to compare the variation constants here exhibited with those for other countries. Greenwood and Brown (1912, p. 43) give means and standard deviations for infant mortality rates for Bavarian communities computed from the data of Groth and Hahn (1910). Taking their longest series (161 observations) and changing from rates per 100 living births, as they state the figures, to rates per 1,000 births to correspond with other data in this paper, we have:

Mean infant mortality rate (1900–1904).... = 243.09
Standard deviation .... = 76.42

It is at once evident that these figures are of about the same order of magnitude as those shown in Table 3 for the colored population of cities in the United States Birth Registration Area. The percentage

TABLE 4.

Analytical constants for infant mortality curves.

Group.	N	ν_1†	μ_2†	μ_3†	μ_4†	β_1	$\sqrt{\beta_1}$	β_2
Cities over 25,000,* Total, 1915	98	2.7245	1.7081	1.1649	9.1772	.2723	.5218	3.1454
" " " " 1916	99	3.6263	1.5245	.3296	7.4924	.0307	.1751	3.2440
" " " " 1917	144	2.4792	1.3746	1.0234	7.7984	.4033	.6350	4.1274
" " " " 1918	144	2.8889	1.5710	1.1084	8.0719	.3168	.5629	3.2706
Cities under 25,000,* Total, 1915	153	4.5490	2.2734	1.5528	19.2617	.1804	.4247	3.4194
" " " " 1916	156	4.7115	2.6219	2.2304	25.6044	.2760	.5254	3.7246
" " " " 1917	236	3.4619	2.2415	3.3963	23.2314	1.0243	1.0121	4.6218
" " " " 1918	236	3.0805	3.5670	9.2407	79.0312	1.8302	1.3529	6.2116
Rural counties, Total, 1915	358	3.6536	1.4336	1.5898	10.6856	.8579	.9262	5.1995
" " " 1916	381	2.7638	1.6824	1.3432	9.5167	.3789	.6155	3.3623
" " " 1917	1127	3.6007	1.6525	1.8928	14.7237	.7939	.8910	5.3916
" " " 1918	1127	3.7214	2.0165	2.4618	17.4078	.7391	.8597	4.2810
Rural counties, White, 1917	232	2.8103	1.4583	1.2374	9.2685	.4937	.7027	5.8472
" " " 1918	234	2.7949	2.0883	2.0117	17.4371	.4444	.6666	3.9986
Rural counties, Colored, 1917	231‡	5.2381	8.2279	33.3269	476.3210	1.9940	1.4121	7.0359
" " " 1918	234	6.8632	10.9407	54.8500	866.4468	2.2973	1.5157	7.2386

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* In 1910.

† It should be expressly noted that the values of the moments ν_1 (about arbitrary origin at the lower range end), and μ_2, μ_3 and μ_4 (about the mean) as here given, are in *units of grouping*, and not in terms of death rates. The unit of grouping, as can be seen in Table 1, is 20 deaths per 1000 births.

‡ These are the constants after dropping the single aberrant observation in the death rate class 600–619 deaths per 1000 births.

as we have already seen, the mode lies to the left of the mean. In many instances (notably in the distributions for the colored population of rural counties) the skewness values rise to considerable magnitudes and may be regarded as significantly different from zero, having regard to their probable errors. In other words, the rate of infant mortality in these different American demographic units tends generally to distribute itself in a substantially unsymmetrical fashion about the mean, extremely high rates occurring more frequently than correspondingly low rates. This fact might perhaps be taken to indicate that the task confronting the administrative control of infant mortality in certain communities of the United States and yet to be accomplished is even greater than what has already been accomplished in the past, great and worthy of commendation as that is.

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" " " " 1917	144	2.4792	1.3746	1.0234	7.7984	.4033	.6350	4.1274
" " " " 1918	144	2.8889	1.5710	1.1084	8.0719	.3168	.5629	3.2706
Cities under 25,000,* Total, 1915	153	4.5490	2.2734	1.5528	19.2617	.1804	.4247	3.4194
" " " " 1916	156	4.7115	2.6219	2.2304	25.6044	.2760	.5254	3.7246
" " " " 1917	236	3.4619	2.2415	3.3963	23.2314	1.0243	1.0121	4.6218
" " " " 1918	236	3.0805	3.5670	9.2407	79.0312	1.8302	1.3529	6.2116
Rural counties, Total, 1915	358	3.6536	1.4336	1.5898	10.6856	.8579	.9262	5.1995
" " " 1916	381	2.7638	1.6824	1.3432	9.5167	.3789	.6155	3.3623
" " " 1917	1127	3.6007	1.6525	1.8928	14.7237	.7939	.8910	5.3916
" " " 1918	1127	3.7214	2.0165	2.4618	17.4078	.7391	.8597	4.2810
Rural counties, White, 1917	232	2.8103	1.4583	1.2374	9.2685	.4937	.7027	5.8472
" " " 1918	234	2.7949	2.0883	2.0117	17.4371	.4444	.6666	3.9986
Rural counties, Colored, 1917	231‡	5.2381	8.2279	33.3269	476.3210	1.9940	1.4121	7.0359
" " " 1918	234	6.8632	10.9407	54.8500	866.4468	2.2973	1.5157	7.2386

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* In 1910.

† It should be expressly noted that the values of the moments ν_1 (about arbitrary origin at the lower range end), and μ_2, μ_3 and μ_4 (about the mean) as here given, are in *units of grouping*, and not in terms of death rates. The unit of grouping, as can be seen in Table 1, is 20 deaths per 1000 births.

‡ These are the constants after dropping the single aberrant observation in the death rate class 600–619 deaths per 1000 births.

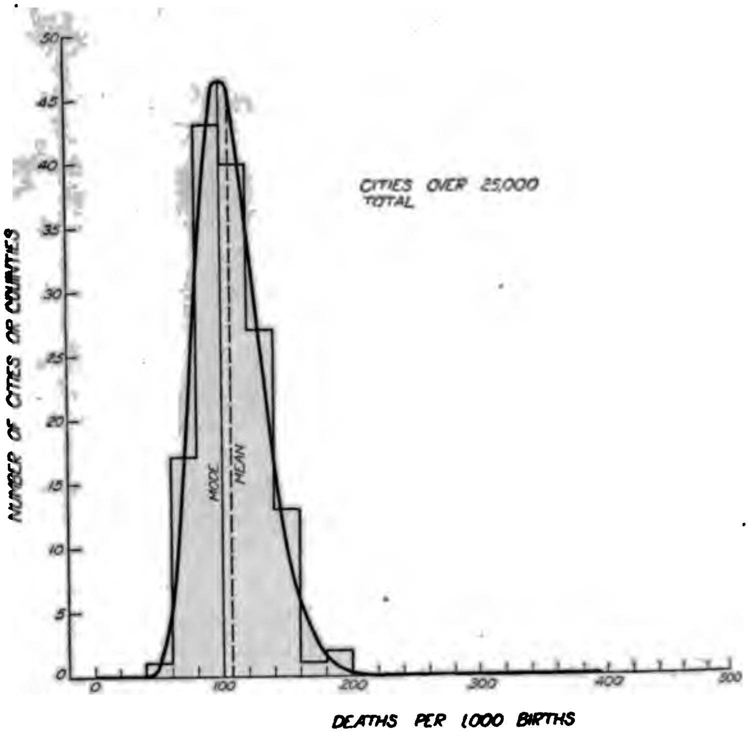

Fig. 3. Frequency histogram and fitted skew curve for variation in th total rate of infant mortality in 1918 in cities of over 25,000 population (in 1910)

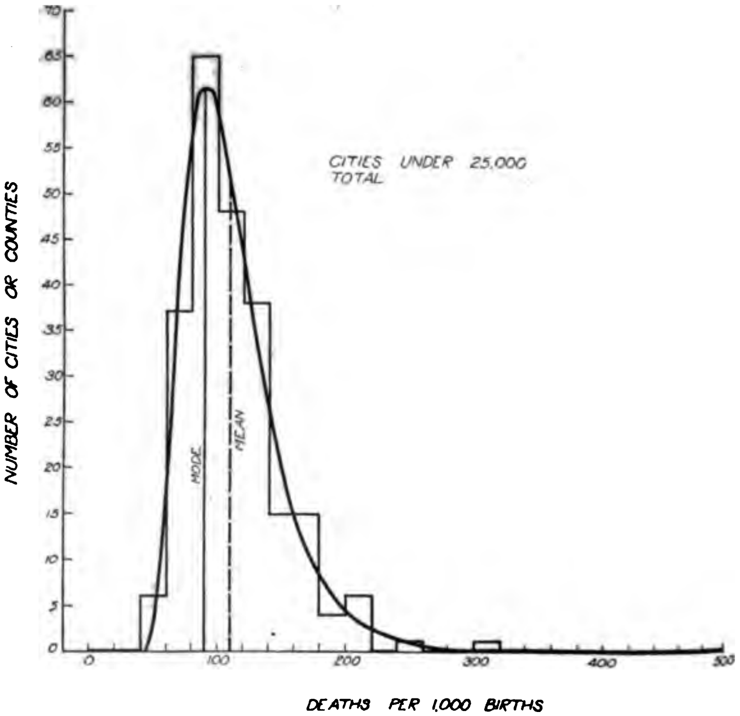

Fig. 4. Frequency histogram and fitted skew curve for variation in the total rate of infant mortality in 1918 in cities of under 25,000 population (in 1910).

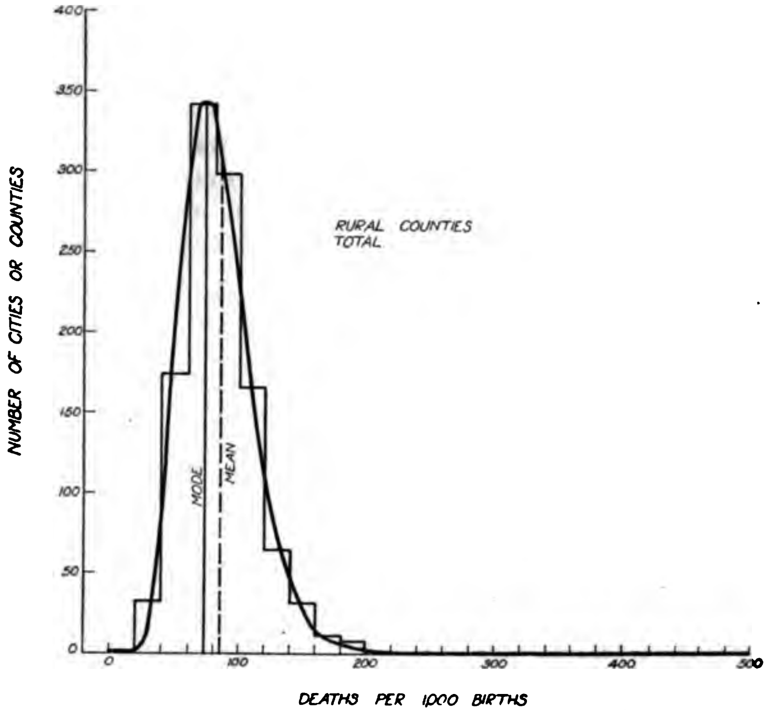

FIG. 5. Frequency histogram and fitted skew curve for variation in the total rate of infant mortality in 1918 in rural counties of the Birth Registration Area.

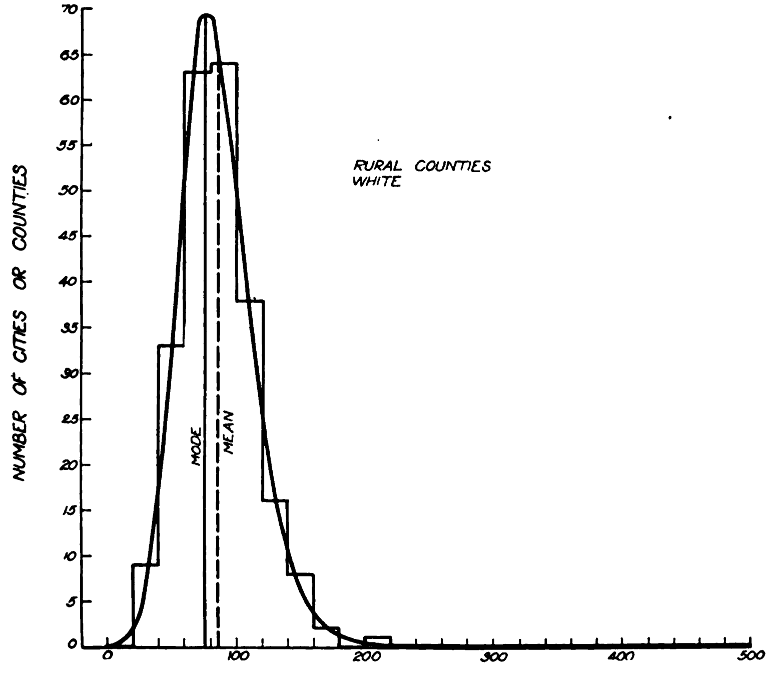

FIG. 6. Frequency histogram and fitted skew curve for variation in the rate of infant mortality among whites in 1918 in rural counties of the Birth Registration Area.

of the standard deviation in the mean for the Bavarian data is 31.44 per cent., indicating that relatively as well as absolutely the degree of variation was about the same in the infant mortality of these Bavarian communities twenty years ago as in our urban colored populations at the present time.

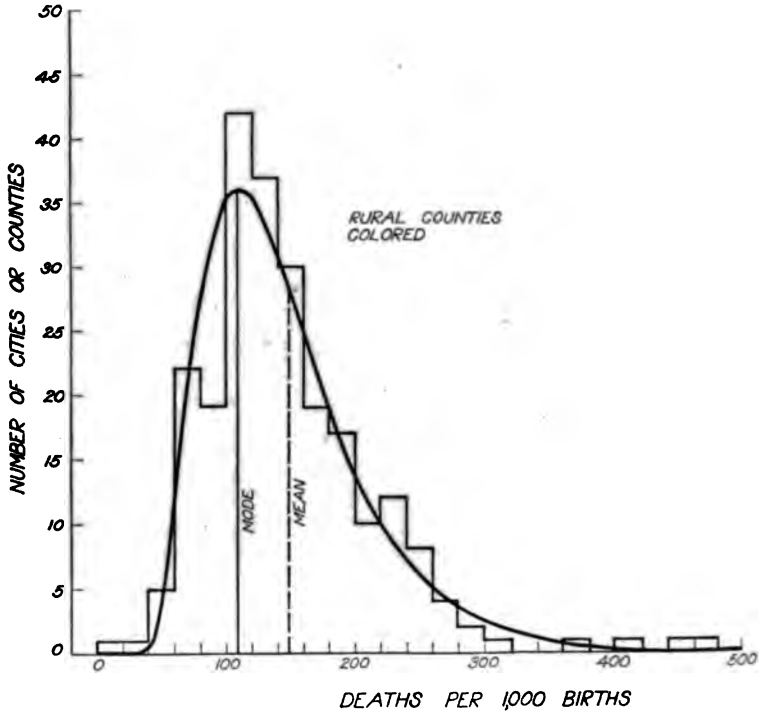

FIG. 7. Frequency histogram and fitted skew curve for variation in the rate of infant mortality among negroes in 1918 in rural counties of the Birth Registration Area.

It has seemed desirable, in the case of certain of the distributions, which may be fairly considered as typical representatives of all, to go on and fit the appropriate skew frequency curves to the observations. The results are shown in Figs. 3–7.

It is evident that the curves in general fit closely the observed facts. The greatest discrepancy between theory and observation is found in Figure 7, the negro rates for rural counties. Here the observations are obviously rough, and probably the curve given is as satisfactory a result as could be obtained with such material.

The equations for the curves are as follows:

Cities over 25,000, total:

$$\text{(Type I)}\quad y = 46.5502\left(1+\frac{x}{2.8946}\right)^{4.4296}\left(1-\frac{x}{14.5155}\right)^{22.2126}$$

Cities under 25,000, total:

$$\text{(Type VI)}\quad y = 8.8238 \times 10^{35}\ (x - 18.4653)^{2.9924}\ x^{-26.7505}$$

Rural counties, total:

$$\text{(Type VI)}\quad y = 9.2560 \times 10^{70}\ (x - 17.7766)^{8.9630}\ x^{-55.2026}$$

Rural counties, white:

$$\text{(Type IV)}\quad y = 0.005609\left[1+\frac{x^2}{(4.5095)^2}\right]^{-12.5407} e^{25.9862\tan^{-1}\frac{x}{4.5095}}$$

Rural counties, colored:

$$\text{(Type VI)}\quad y = 4.2887 \times 10^{27}\ (x - 20.9629)^{3.0627}\ x^{-19.9804}$$

CONCLUSIONS.

The facts which are set forth in this paper, being wholly objective, and based upon data which are in the aggregate highly accurate—being simply registered births as a whole and registered deaths under one year of age as a whole, both registrations being subject at every step to close control and scrutiny by both local and federal officials—lead to certain definite and clean-cut conclusions, to wit:

1. Infant mortality rates exhibit a relatively high degree of place variation in both rural and urban areas of the United States. This high variation is evident upon any and every basis of estimation which may be chosen, whether range, standard deviation, or coefficient of variation.

2. This great variation demonstrates the opportunity which still exists for effective administrative control and reduction of infant mortality.

3. The distributions of place variation in infant mortality are unimodal for both urban and rural, white and colored populations.

4. There was no certainly significant decline in the mean or average rate of infant mortality in the United States Birth Registration Area during the years 1915–18, inclusive.

5. Presumably as a result of the influenza epidemic the mean infant mortality rates were higher (in some cases significantly so) in 1918 than in 1917 in cities. No such difference is exhibited by the mean rates for rural areas.

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY THE BIBLIOGRAPHIC SERVICE, JUNE 27

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL

XVIII. FURTHER OBSERVATIONS ON THE ANATOMICAL BASIS OF FECUNDITY[1]

RAYMOND PEARL AND WILLIAM FREEMAN SCHOPPE

TWO FIGURES

INTRODUCTION

Some eight years ago one of us[2] published the results of some counts of the visible oöcytes upon the ovaries of domestic fowls. The number of birds dealt with at that time was statistically small, amounting to but seventeen. The problem to which attention was directed in this earlier work was stated as follows:

"To what extent are observed variations in fecundity (i.e., in the number of eggs laid) to be referred to anatomical differences? In other words, does the ovary of a high-producing hen, with, for example, a winter record of from 75 to 115 eggs, contain a larger number of oöcytes than does the ovary of a hen which is a poor producer, laying no eggs in the winter period and perhaps but 10 or 15 eggs in the year?"

The purposes of the present work were to extend the observations to a larger number of birds; to check the general reliability of the results by getting independent counts of another observer; to get data on wild birds, and to carry out certain experiments on ovarian regeneration. The counts of the present series will be combined with the earlier data and the whole submitted to certain biometrical treatment.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, no. 24.

The observations on which this paper is based were made some years ago at the Maine Agricultural Experiment Station. The paper has been completed by the senior author.

[2] Pearl, R. The mode of inheritance of fecundity in the domestic fowl. Jour. Exp. Zool., vol. 13, pp. 153-268, 1912.

In dealing, in this and the earlier work referred to, with oöcytes visible to the unaided eye alone, the tacit assumption is that the number of such visible ova is correlated to a high degree with the undetermined—and indeed undeterminable—total number of ova in the ovary. The work of von Hanseman[3] and of Käppeli[4] indicates that this assumption is justified. Occasionally a case turns up, as will be shown presently in detail, where the physical condition of the bird's ovary as a whole greatly raises the limit of oöcyte visibility, so to speak. This condition, however, we have found to occur but once in nearly forty birds examined from this point of view. So far as concerns the remainder of the counts, we are convinced, after direct experience with the material, that the ratio between visible ova and mass of the ovary as a whole, and by inference the number of invisible ova, is normally a rather constant one, and that consequently our material is as homogeneous and trustworthy as any statistical data on the soft parts of animals.

Regarding the method of counting, the following may be added to what was said in the earlier paper (loc. cit., p. 296).

The ovaries, when taken from the birds, were placed in a 10 per cent solution of formalin, in order to preserve them until counts could be made. In making the earlier counts, fresh ovaries were at first used, but later after trying various preservatives it was concluded that the use of formalin was most satisactory, as it preserved the ovaries in perfect condition and made counting possible at any time.

The functioning ovary when taken from the bird closely esembles a bunch of grapes. It is made up of a large number of oöcytes held together by connective tissue, joined to a heavy talk by which they are held in place in the body.

The oöcytes are of various sizes, and for convenience in countng four divisions were made: *a*) follicles from which the ova have been discharged; *b*) oöcytes over 1 cm. in diameter; *c*) those over mm. and less than 1 cm.; *d*) those less than 1 mm.

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[3] Hansemann, D. Ueber den Kampf der Eier in den Ovarien. Arch. f. Entwicklungsmech., 1912, Bd. 35, S. 223–235, 1 pl.

[4] Käppeli, I. Beiträge zur Anatomie und Physiologie der Ovarien von wildlebenden und gezähmten Wiederkäuern und Schweinen. Bern, 1908, K. J. Wyss. 78 pp., 5 tab., 6 pl.

Since the first class indicates the number of discharged ova, it theoretically ought to conform closely with the total number of eggs laid. However, as the follicles are being constantly absorbed they eventually reach a stage where it is difficult to determine positively if they are follicles or parts of connective tissue. It was practically impossible to measure every oöcyte, consequently the last three divisions are approximate, but conform very closely, since, if there was any doubt, the oöcyte was measured.

In making the counts small pieces of the ovary were separated, placed in a dish of water, and teased apart in order to distinguish the ova. Only such ova were counted as could be seen with the unaided eye. A low-power dissecting lens was used in this work, not to find more ova, but to assist in more readily and accurately counting those that could be seen.

DATA

In table 1 are collected together all the data collected at the Maine Station on the number of visible oöcytes in the ovaries of birds, including the counts of the earlier paper as well as those of the present investigation. The old counts are designated by asterisks. Within each breed the birds are arranged in descending order of total number of visible oöcytes.

A word should be said concerning cases 25, 26, 27 and 28. The two Pekin ducks were both in a healthy condition and ovaries were apparently functioning. In the case of no. 26 this was certain, since an egg was found in the uterus. In case of no. 28, a Toulouse goose, the ovary seemed to be in a very unhealthy condition, as all the larger and some of the smaller oöcytes were being absorbed. The tissue was very tender and several oöcytes were broken in removing the ovary from the body and in placing it in the preservative, so that although the ovary is credited with two follicles it is doubtful if the bird ever laid an egg.

Case no. 27, a young goose, hatched in the spring of 1912, proved a very desirable bird. She had a large ovary (199 grams) in a very active stage of production. An egg was found in the oviduct. The eleven oöcytes over 1 cm. in diameter ranged in weight from 2 to 32 grams. The five largest weighed 32, 31.5,

TABLE 1

Showing the number of visible oöcytes in the ovary of certain birds

CASE NUMBER	BREED OR SPECIES	DATE HATCHED	DATE KILLED	TOTAL EGGS LAID	WINTER PRODUCTION	DISCHARGED FOLLICLES	OÖCYTES OVER 1 CM. IN DIAMETER	OÖCYTES 1 MM. TO 1 CM. IN DIAMETER	OÖCYTES UNDER 1 MM. IN DIAMETER	TOTAL VISIBLE OÖCYTES
	A. Domestic fowl (Gallus sp.)									
*1	Barred Plymouth Rock	Apr. 28, '10	Apr. 4, '11	34	3	49	7	29	2121	2306
2	Barred Plymouth Rock	Spring, '07	May, 14, '10	412	82	31	7	70	2191	2299
*3	Barred Plymouth Rock	June 2, '10	Mar. 24, '11	15	0	17	6	49	2029	2101
4	Barred Plymouth Rock	May 11, '10	Nov. 2, '12	205	43	99	2	136	1775	2012
*5	Barred Plymouth Rock	June 2, '10	Mar. 30, '11	10	0	12	7	51	1596	1666
6	Barred Plymouth Rock	June 1, '10	Nov. 4, '12	63	4	74	0	297	1275	1646
*7	Barred Plymouth Rock	May 19, '10	Mar. 17, '11	19	5	24	5	92	1455	1576
*8	Barred Plymouth Rock	Mar. 30, '09	July 7, '10	23	0	21	12[1]	142	1346	1521
9	Barred Plymouth Rock	Mar. 28, '11	Sept. 29, '11	5		18	8	57	1274	1357
*10	Barred Plymouth Rock	June 1, '10	Mar. 28, '11	10	3	17	9	53	1149	1228
*11	Barred Plymouth Rock	June 2, '10	Mar. 24, '11	16	0	23	6	42	1123	1194
*12	Barred Plymouth Rock	June 2, '10	Mar. 14, '11	17	5	12	8	68	1096	1174
*13	Barred Plymouth Rock	June 1, '10	Mar. 10, '11	7	0	8	5	62	839	914
*14	White Leghorn	May 28, '09	Dec. 15, '10	197	32	217	1	108	3279	3605
*15	White Leghorn	May 18, '09	Dec. 20, '10	198	54	75	[2]	231	2146	2452
*16	White Leghorn	June 14, '09	Dec. 22, '10	2	0	43	[2]	80	2022	2145
*17	White Leghorn	May 21, '09	Dec. 13, '10	10	0	11	[2]	75	1626	1701
*18	Cornish Indian Game	Apr. 21, '09	July 12, '10	52	13	54	6	167	1328	1550
19	Light Brahma	?	May 25, '12	?	?	28	9	119	1074	1230
20	F_1 Cross-bred	May 2, '11	Apr. 9, '12	46	30	47	6	111	2622	2786

21	F₁ Cross-bred	Apr. 26, '11	June 14, '12	143	73	52	18	52	2418	2540
22	F₁ Cross-bred	Apr. 21, '11	June 14, '12	91	38	42	5	43	2239	2529
23	F₁ Cross-bred	Mar. 29, '11	June 26, '12	48	17	40	7	46	2128	2221
*24	F₁ Cross-bred	Mar. 31, '10	Mar. 20, '11	124	106	50	5	70	1875	2000
		B. Other domestic birds								
25	Pekin duck	?	June 12, '12	?	?	10	19	276	892	1197
26	Pekin duck	Spring, '12	Mar. 27, '13	?	?	16	5	141	777	939
27	Toulouse goose	Spring, '12	Apr. 9, '13	?	?	12	11	129	1770	1922
28	Toulouse goose	Spring, '12	Apr. 8, '13	?	?	2	18	55	982	1057
*29	Guinea-hen	?	Jan. '11	?	?	9	3	36	717	765
*30	Guinea-hen	?	Jan. '11	?	?	0	3	38	545	586
		C. Wild birds								
31	Crow (Corvus brachyrhynchus)	?	July 10, '12	?	?	0	0	0	808	808
32	Crow (Corvus brachyrhynchus)	?	Apr. 11, '12	?	?	0	0	13	708	721
33	Kingbird (Tyrannus tyrannus)	?	Apr. 27, '12	?	?	0	0	0	505	505
34	Kingbird (Tyrannus tyrannus	?	Apr. 28, '12	?	?	0	0	0	247	247
35	Meadow-hawk	?	Apr. 8, '12	?	?	0	0	0	102[3] 135[4]	237

[1] This includes eight yolks in process of absorption.

[2] Bird not in laying condition when killed.

[3] Right ovary.

[4] Left ovary.

27, 23.5 and 21 grams. The last six weighed 14, 12.7, 12, 8, 3.5, and 2 grams, and seemed to constitute a series which coincided closely with weights taken of the larger öocytes in hens.

Theoretically, the number of discharged follicles and the total number of eggs laid should agree. Actually, this condition is never absolutely realized in the counts. In some cases it will be found that the count of follicles is greater than the total eggs laid. This can be accounted for in four ways: *a*) the bird may have laid while still on the range or before she was put in the laying house; *b*) eggs may have been laid on the floor before the birds became accustomed to using the nests; *c*) some of the yolks may have missed the oviduct, being deposited in the body cavity, and eventually absorbed; *d*) in some cases pieces of projecting connective tissue may have been mistaken for a follicle in making the counts. In other cases it will be seen that the eggs laid exceeded the number of follicles found. This is directly due to not being able to distinguish the follicles after they had become nearly or completely absorbed.

In addition to the thirty-five counts listed in table 1, the oöcytes of one other F_1 cross-bred bird were counted on a somewhat different plan. The ovary presented an extraordinary discreteness of separation of even minute oöcytes. In this case a low-power dissecting lens was used in the counting, and all oöcytes were enumerated which could be counted with the aid of the lens. The results were as follows:

Case No. 36

Lens aided count

F_1 crossbred

Hatched April 21, 1911
Killed June 14, 1912
Total eggs laid = 69
Winter production = 46
Discharged follicles = 29
Oöcytes > 1 cm. = 2
Oöcytes 1 mm. to 1 cm. = 80
Oöcytes < 1 mm. = 13,365
Total oöcytes = 13,476

This case shows the enormous difference which the physical construction of the ovary may make in the number of visible oöcytes in exceptional cases. Since this condition has been seen but this once in many hundreds of fowl ovaries examined, it cannot be considered in any way to invalidate the counts of normal ovaries (in the sense of visibility of oöcytes) given in table 1.

With the data before us, we may proceed to their biometrical analysis along certain lines.

TABLE 2

Constants deduced from table 1

BREED OR SPECIES	MEAN NUMBER OF VISIBLE OÖCYTES	STANDARD DEVIATION	COEFFICIENT OF VARIATION
White Leghorn fowls	2476	705	28.5
F_1 Cross-bred fowls	2415	274	11.4
Barred Plymouth Rock fowls	1615	433	26.8
All fowls counted	1906	615	32.3
Toulouse geese	1489	432	29.0
Pekin ducks	1068	129	12.1
All domestic water-fowl counted	1279	382	29.9
Guinea-hens	676	90	13.3
Crows	764	44	5.8
Kingbirds	376	129	34.3
All wild birds counted	504	235	46.7
All wild birds and guinea-hens	553	219	39.6

BREED AND SPECIES COMPARISONS

The first point for consideration is the relation of the number of visible oöcytes to laying capacity in the different breeds. It is evident enough from simple inspection of table 1 that there is no correlation among individual fowls between visible oöcytes and egg production. The case may, however, be different when the comparison is made between breeds. As a necessary preliminary to the discussion of this point, table 2 is presented, containing the required biometric constants.

It is evident from table 2 that, considering broad categories, the mean number of visible oöcytes falls in the same order as the normal egg production of the kind of bird concerned. The domestic fowls stand at the head of the birds considered in respect of fecundity and also in number of visible oöcytes. The wild birds (including the guinea-hens) stand at the bottom of the list in both respects, while the domestic water-fowl occupy an intermediate position in both respects. The guinea-fowls are included in the average with wild birds for the reason that in its reproductive activities this creature behaves in all essentials like a wild bird. It refuses to lay except in a hidden nest of its own choosing, and lays in the course of a year a single clutch of not more than fourteen to sixteen eggs.[5]

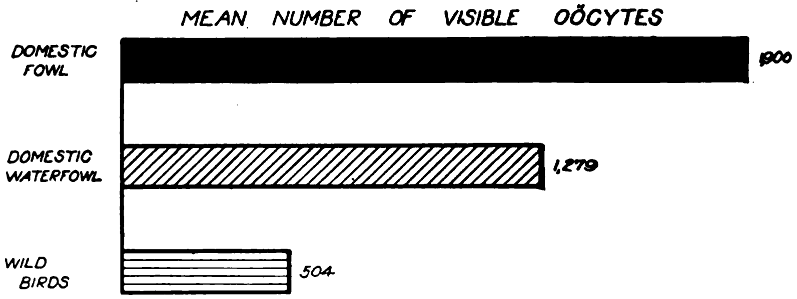

Fig. 1 Showing average number of visible oöcytes on ovary of, *a*) domestic fowl, *b*) domestic waterfowl, and, *c*) wild birds.

The relations of the three group averages discussed are shown graphically in figure 1.

It is of interest to note the considerably higher average number of visible oöcytes in the F_1 cross-breds than in the pure Barred Plymouth Rocks. All of these cross-breds were genetically half Barred Plymouth Rock, of the same strain as the pure-bred birds furnishing the counts and half from some other breed of lower fecundity than the Barred Rocks. The fact that the F_1 birds show a relatively high ovarian count, roughly 20 per cent higher than the average of the more fecund parent strain, suggests that we may have here a result due to heterosis, of the same sort essentially as those which have been observed particularly by East[6] in plants.

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[5] These statements apply, from personal observation, certainly to the strain of guinea-fowls from which the birds here counted came.

The coefficients of variation for these ovarian counts fall, in the longer and hence more reliable series, in the neighborhood of a value of 30 per cent. This is about the value found for the relative variability of a number of physiological characters, particularly fecundity of the domestic fowl as measured by annual egg production.[7]

RELATION OF OÖCYTE NUMBER TO AGE

It is a well-known fact that in the domestic fowl egg production rapidly diminishes after the first year or in some breeds such as the Leghorns the first two years of life. Fowls may live for a considerable number of years, but generally after the early years they lay no eggs at all, or at most one small clutch in the spring.

In the case of Barred Plymouth Rocks, of the Maine Station strain, egg production usually practically ceases after the bird is two years old. It is commonly assumed that this cessation or great reduction in fecundity with age is due to atrophic processes in the ovary. It is, on this account, of interest to see in what manner the counts of visible oöcytes change with age.

Unfortunately, the material in which the point can be tested is meager. The Barred Plymouth Rocks constitute the only group sufficiently large, and there is a highly uneven distribution by age. Of the thirteen birds, nine were under one year of age when killed, one was between one and two, two between two and three, and one between three and four. It will be worth while, however, to see what this admittedly meager material shows. The data are exhibited in table 3 and graphically in figure 2.

In so far as the data may be trusted as fairly representative of what generally occurs, they indicate that the number of visible oöcytes increases with age, within the age limits of the observations. Apparently what happens in the ovary of the fowl after the bird reaches laying age is that ova are constantly migrating to the surface and developing thereon up to something under 1 mm. in diameter. Of these a much smaller number continue their growth and are finally laid. Since the number developing up to the 1-mm. size, however, always greatly exceeds in any given time unit the number developing farther to laying size, and since this disproportion may be reasonably assumed to become progressively greater as laying activity diminishes, which it is

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[6] Cf. East, E. M., and Jones, D. F. Inbreeding and outbreeding. 1919.

[7] Cf. Pearl, R., and Surface, F. M. A biometrical study in egg production in the domestic fowl. I. Variation in annual egg production. U. S. Dept. of Agric., Bur. of An. Ind. Bull. 110, Part I.

TABLE 3

Mean number of visible oöcytes in Barred Plymouth Rocks of different ages

AGE CLASS IN YEARS	MEAN AGE OF GROUP IN MONTHS	NUMBER OF BIRDS	MEAN NUMBER OF VISIBLE OÖCYTES
Under 1	9.48	9	1502
1 to 1.9	15	1	1521
2 to 2.9	29.25	2	1829
3 to 3.9	37	1	2299

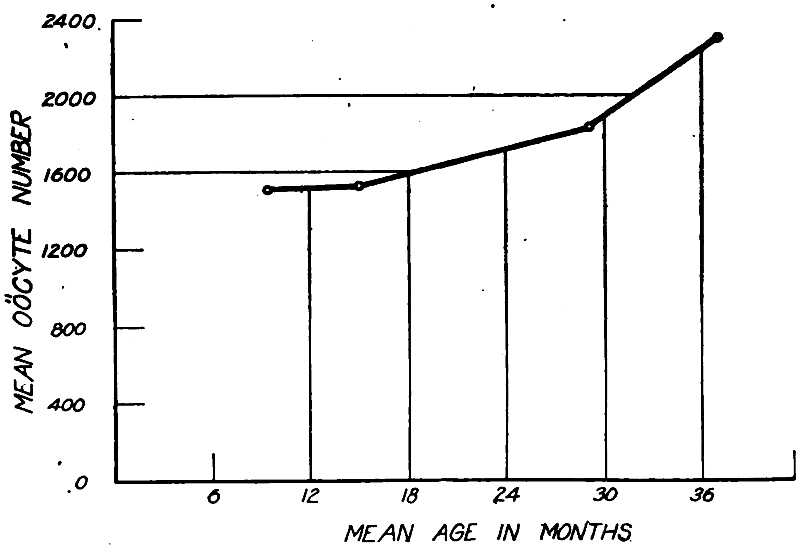

Fig. 2 Showing the change in mean number of visible oöcytes with advancing age in Barred Plymouth Rocks.

known to do with advancing age, and since, as table 1 shows, the small oöcytes under 1 mm. in diameter make up the vast majority of all those visible, it follows that we should expect to find more and more visible oöcytes as age advances up to an asymptote which represents the complete cessation of all ovarian activity. Presumably, the oldest of the birds here counted was not old enough to have reached this condition of complete ovarian rest. Our general knowledge of ovarian functioning in fowls, from personal observation, would lead to this last conclusion as well as the form of the curve in figure 2.

Probably the complete curve for visible oöcytes on an age base, if we had data to plot it, would be of the shape of a flattened S, with an upper asymptote, representing complete ovarian rest, and a lower zero asymptote representing no visible oöcytes at an early age. This is a form of curve which has been found to represent growth phenomena of various sorts.[8]

EXPERIMENTS ON OVARIAN REGENERATION

It is well known that the ovary in birds is an organ that regenerates readily and freely after injury. Indeed, this fact constitutes one of the chief technical difficulties in making a complete and permanent castration of female birds. At the same time it has been held as a basic biological doctrine that during the life of the individual there neither is nor can be any increase in the number of primary oöcytes beyond those originally laid down when the ovary is formed. It occurred to us to make a quantitative study of visible oöcytes in regenerated fowls' ovaries to determine to what extent an increase could be brought about in the number of primary oöcytes which develop to visible size at the surface of the ovary. Other experiments had inducing regeneration of the ovary a bird could more eggs than she would have had there be with the ovary. The present experiments we a quantitative test of this suggested result.

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[8] Robertson, T. Brailsford. On the normal rate of growth of an individual and its biochemical significance. Arch. für Entwickelungsmechanik der Organismen, Bd. 25, S. 581–514.

Robertson, T. Brailsford. Further remarks on the normal rate of growth of an individual, and its biochemical significance. Arch. für Entwickelungsmechanik der Organismen, Bd. 26, S. 108–118.

Pearl, R., and Reed, Lowell J. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci., vol. 6, no. 6, 1920.

Eight birds were used in the experiments, outh Rocks and four Cornish Indian Games protocols follow:

Bird no. 9017, operation 131. Bird in fin yolks being absorbed; about five-sixths of th with almost no loss of blood.

Bird no. 9030, operation 129. Bird not in Largest yolk about 8 mm. in diameter. Ovar condition: about two-thirds was removed.

Bird no. 9026, operation 128. Bird in large yolks present; one-half of ovary removed. siderable bleeding.

Bird no. 9022, operation 127. Bird in nearl but had stopped temporarily. Large yolks of ovary removed. There was considerable ble

Bird no. 9018, operation 125. Bird ap Ovary in very bad condition. Large number and yolk tumors. Evidently septic condition surface was thick and opaque. Removed la and all tumors. There was considerable bleedi

Bird no. 9023, operation 123. Bird in h Ovary not in laying condition; about two-third

Bird no. 9019, operation 126. Bird in h Ovary healthy, but in a non-laying conditio removed than in either 127, 125, or 123.

The pieces of removed ovary were placed in fo later made.

It was the original intention to keep these bi all started to lay, but this was found impossi of time. As it was, half of the birds had sta was only a question of time before the others all the ovaries were in a very healthy condition

Bird no. 9017 was the only one that died. An autopsy showed that the ovary was in an apparently normal condition. There were three absorbing yolks. The oviduct was normal, and as the bird had been laying her death could not be attributed to the operation.

Bird no. 9030 was killed to secure the ovary. The autopsy showed her to be in a very healthy condition. There were four large normal yolks in the ovary and one absorbing yolk. There was also an egg in the oviduct.

Bird no. 9026, killed to secure ovary. She was in a normal healthy condition. There were three large yolks in the ovary and an egg in the oviduct. Ovary of fair size, but not as large as in a normal bird.

Although bird no. 9022 had not laid, she was killed. It was found that the ovary was in a laying condition, containing five large yolks. Several yolks were found in the body cavity surrounded by adhesions. There was no apparent reason for this, as the oviduct seemed to be normal. The funnel was in its normal position and was not injured.

Bird no. 9018. It will be recalled that at the time of the operation this bird had a very bad ovary, containing several absorbing yolks and yolk tumors. When killed, although she had not laid, it was found that the ovary had several enlarging yolks and that the oviduct was enlarging. There were no tumors or septic tissue. Evidently the operation had a beneficial effect.

Bird no. 9023 was the only one whose ovary showed no apparent growth. Both the ovary and oviduct were in a healthy condition, but small.

The two days preceding the day on which bird no. 9019 was killed she layed. Her ovary was found to contain two large normal yolks and one absorbing yolk.

Bird no. 9025 did not lay; when killed the ovary was found to be healthy. It was enlarging, as was also the oviduct.

Counts were made in the usual way of *a*) visible oöcytes on the portion of ovary removed at the original operation and, *b*) visible oöcytes on the regenerated ovary at the death of the bird.

The results of these counts together with other pertinent data are given in tables 4 and 5.

The data of tables 4 and 5 are brought togetl in table 6, which gives in sum the total oöcytes

TABLE 4

Showing number of visible oöcytes in part of ovary rem operation

BIRD NUMBER	BREED	DATE OF HATCHING	DATE OF OPERATION	TOTAL EGGS LAID BEFORE OPERATION	DISCHARGED FOLLICLES	OÖCYTES OVER 1
9017	B. P. R.	3/30/11	9/12/12	124	26	7
9030	B. P. R.	3/10/11	9/12/12	125	7	1
9026	B. P. R.	4/ 1/11	9/12/12	125	29	4
9022	B. P. R.	3/29/10	9/12/12	79	17	1
9018	C. I. G.	5/19/10	9/10/12	42	14	7
9023	C. I. G.	6/ 2/10	9/10/12	35	16	0
9019	C. I. G.	3/31/10	9/10/12	26	9	0
9025	C. I. G.	4/ 1/10	9/10/12	31	12	0

TABLE 5

Showing number of visible oöcytes in ovary of operated

BIRD NUMBER	BREED	DATE OF HATCHING	DATE OF KILLING	DATE OF OPERATION	FIRST EGG LAID AFTER OPERATION	TOTAL EGGS LAID AFTER OPERATION	DIS-CHAR ED FOLL CLES
9017	B. P. R.	3/30/11	2/24/13	9/12/12	2/17/13	13	.10
9030	B. P. R.	5/10/11	3/31/13	9/12/12	1/11/13	25	9
9026	B. P. R.	4/ 1/11	3/31/13	9/12/12	3/27/13	2	11
9022	B. P. R.	3/29/10	4/ 2/13	9/12/12		0	1
9018	C. I. G.	4/19/10	4/ 3/13	9/10/12		0	0
9023	C. I. G.	6/ 2/10	4/ 3/13	9/10/12		0	0
9019	C. I. G.	3/31/10	4/ 3/13	9/10/12	4/ 1/13	2	7
9025	C. I. G.	4/ 1/10	4/ 3/13	9/10/12		0	2

From table 6 it is seen that in all cases exc 9018) the number of visible oöcytes on the pi smaller than the number found on the rege

TABLE 6

Showing total number of visible oöcytes in ovary of operated birds

BIRD NUMBER	BREED	DATE OF HATCHING	DATE OF KILLING	DATE OF OPERATION	FIRST EGG LAID AFTER OPERATION	TOTAL NUMBER LAID IN LIFE	DISCHARGED FOLLICLES	DIAMETER OF OÖCYTES			TOTAL VISIBLE OÖCYTES
								Over 1 cm.	1 cm. to 1 mm.	Under 1 mm.	
9017	B. P. R.	3/30/11	3/24/13	9/12/12	2/17/13	124	26	7	119	854	1006
						13	10	3	94	1874	1981
					Total	137	36	10	213	2728	2987
9030	B. P. R.	5/10/11	3/31/13	9/12/12	1/11/13	125	7	1	75	238	321
						25	9	5	122	1197	1333
					Total	150	16	6	197	1435	1654
9026	B. P. R.	4/ 1/11	3/31/13	9/12/12	3/27/13	125	29	4	67	623	723
						2	11	5	100	1435	1551
					Total	127	40	9	167	2058	2274
9022	B. P. R.	3/29/10	4/ 2/13	9/12/12		79	17	1	109	476	603
							1	7	98	977	1083
					Total	79	18	8	207	1453	1686
9018	C. I. G.	5/19/10	4/ 3/13	9/10/12		42	14	7	97	1131	1249
								2	92	936	1030
					Total	42	14	9	189	2067	2279
9023	C. I. G.	6/ 2/10	4/ 3/13	9/10/12		35	16		77	701	794
									73	1125	1198
					Total	35	16		150	1826	1992
9019	C. I. G.	3/ 3/10	4/ 3/13	9/10/12	4/ 1/13	26	9		102	1265	1376
						2	7	3	120	1977	2107
					Total	28	16	3	222	3242	3483
9025	C. I. G.	4/ 1/10	4/ 3/13	9/10/12		31	12		140	780	932
							2	1	177	1578	1758
					Total	31	14	1	317	2358	2690

death, as would be expected. The operation protocol on bird no. 9018 shows that the ovary was in a diseased condition at the time of operation, but at the time of killing the ovary was in normal condition. The protocol states that a "large part of ovary was removed." So far as could be estimated, a larger portion of the ovary was removed at operation in this case than in any other, with the possible exception of 9017.

In order to make clearer comparisons, table 7 has been calculated from the data of table 6.

TABLE 7

Constants from regeneration experiments

GROUP	TOTAL VISIBLE OÖCYTES		
	Mean	Standard deviation	Coefficient of variation
Portion removed—Barred Plymouth Rocks	663 ± 83	246	37.1
Regenerated ovary—Barred Plymouth Rocks	1487 ± 110	328	22.1
Total—Barred Plymouth Rocks	2150 ± 183	543	25.2
Normal Barred Plymouth Rocks (ex. table 2)	1615 ± 81	433	26.8
Portion removed—Cornish Indian Game	1088 ± 79	234	21.5
Regenerated ovary—Cornish Indian Game	1523 ± 146	432	28.3
Total	2611 ± 189	561	21.5
Normal Cornish Indian Game (ex. table 2)	1550[1]		

[1] One case only.

From these data a number of points of interest appear. In the first place, it is evident that the number of visible oöcytes on the regenerated ovaries is substantially the same as the mean number on the normal unoperated ovary of the same breed. In the case of the Barred Rocks the difference is $1615 - 1487 = 128$, an obviously insignificant difference. In the Cornish Indian Games the difference is even smaller, amounting to $1550 - 1523 = 27$. In the latter case we have, unfortunately, only a single normal count, rather than a normal mean.

If we add to the number of oöcytes found upon the regenerated ovary those upon the piece removed at operation to get the total number of visible oöcytes the bird produced in its life, we get a considerable excess over the number produced by normal unoperated ovaries of the same bird. This excess amounts in the case of the Barred Rocks to 2150 − 1615 = 535, an increase of 33 per cent in operated over normal, and in the case of the Cornish Indian Games to 2611 − 1550 = 1061, an increase of 68 per cent in operated over normal. The excess shown by the Barred Rocks can hardly be regarded as statistically significant, on the basis of the present short series. The fact, however, that both breeds show such a consistent set of results throughout is evidence that with a longer Barred Rock series we should get essentially the same result as with the present one and with sufficiently reduced probable errors to make the difference statistically significant. A fair and instructive comparison can be made by lumping together the Barred Rock and Game operated series, and putting the mean total number of visible oöcytes produced during life against the mean total number produced by all normal unoperated fowl, excluding the White Leghorns, since our previous results show that this breed has evidently as a breed character a distinctly larger number of visible oöcytes than any of the other breeds dealt with. On this basis we have:

Mean total visible oöcytes produced during life by eight operated birds	2381±143
Mean total visible oöcytes produced during life by twenty normal birds	1793± 79
Difference	588±163

Here we see that the difference is 3.6 times its probable error, and hence to be regarded as almost certainly statistically significant.

Altogether it is believed that we may safely conclude that by inducing by operative means ovarian regeneration in the domestic fowl the total number of primary oöcytes which develop to visible size is significantly increased.

Following these experiments the senior author undertook much more extensive work on repeated ovarian regeneration. In a series of birds of known and highly interesting genetic constitution (F_2 birds of a carefully studied cross) a considerable portion of the ovary was removed each autumn for four years. The laying record was kept over this long period, and also careful record made of the chicks which developed from oöcytes produced by these regenerated ovaries. Unfortunately, all the records of this experiment were lately destroyed by fire, and hence cannot be reported. The writer regarded this experiment as perhaps the most interesting and fundamentally significant for the problem of genetics of any of his work, with poultry. Mention is made of the matter here in the thought that possibly some one so situated as to be able to carry on work of this sort, which the writer no longer is, may care to repeat the work.

SUMMARY

The chief results of this work may be stated as follows:

1. Detailed counts are given of the number of visible oöcytes on the ovaries of thirty-six birds, including various breeds of domestic fowls, water-fowl, and wild birds.
2. In general the mean number of visible oöcytes on the ovaries of different kinds of birds reflects the normal fecundity or laying activity of the same kinds of birds. This relation does not hold with any exactitude or regularity for differences in fecundity between individual birds.
3. In Barred Plymouth Rock fowls the number of visible oöcytes increases with advancing age of the bird, within the age limits of six months and thirty-seven months. The probable explanation of this phenomenon is given.
4. By removing a portion of the ovary and causing it to regenerate, the total number of oöcytes developing to visible size in the lifetime of the bird is caused to increase from 33 to 68 per cent over the number which develop in the normal, unoperated bird. This increase is shown to be statistically significant.

THE VITALITY OF THE PEOPLES OF AMERICA

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THE VITALITY OF THE PEOPLES OF AMERICA.*

By RAYMOND PEARL.

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I.

The General Problem of Racial Amalgamation.

Science tries ever to peer into the future. Reasoning from the solid basis of human experience in general, that like antecedents are followed by like consequences, it is the constant endeavor of science so to formulate, classify and codify the relations which have in the past subsisted between antecedents and consequences as to be able to pick out the sets which show the maximum degree of constancy, with the thought that then the future may be predicted, if not with absolute certainty, at least with a high degree of probability. Nowhere is the desire to estimate what the future has in store stronger than in the case of matters relating directly to human actions, emotions and thoughts. We know that we are on our way. But where are we going?

This general interest of man in his future social, physical, and mental evolution crystallizes in a specific way for us here and now in the problem of the future of American civilization. That magnificent experiment in human evolution which is called the United States of America has, from its beginning, been at once the embodiment of the loftiest hope and the deep despair of different groups of people. In theory it has always offered what is perhaps the greatest boon intelligent human beings ever crave, equality of opportunity.

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The ideal of freedom of opportunity has probably been as consistently maintained in fact, as would in any case be possible, human nature being what it is. Our gates have been open, with some slight and on the whole insignificant exceptions, to all and sundry. Good, bad and indifferent people have been free to settle here, and have made the fullest use of the freedom. Meantime the country has prospered mightily in material ways, and not insignificantly in things of the spirit, such cheerful pessimists as Mr. Mencken to the contrary notwithstanding. What vexes the mind is the consideration of the question as to whether the abounding blessings which on the whole have associated themselves with our country have appeared because of, or in spite of, the nearly complete abandon with which all kinds of people have been permitted, indeed encouraged, to join in the enterprise. According to one's views as to the answer to this question he is either fairly satisfied or somewhat disturbed as he looks towards the future.

The essential problem with which the present discussion has to do is that of the racial composition, and biostatistical characteristics in general, of the probable future population of this country. Our judgment on this problem must be determined by a careful examination of what has happened in the past together with what is now going on.

Our problem is purposely stated in this way to emphasize the fact that basically the problem of the future of America is simply one restricted phase of the general problem of population. Whether racial amalgamation or fusion occurs or not, gross population growth goes on steadily. Strange, and indeed inconceivable, as it may appear to many persons, the United States is going to be sometime a densely crowded country, where each individual, consciously or unconsciously, will be challenging the right of every other individual to existence. And, by every present indication, the time when this pressure of population will be definitely felt in this country is not so far away. Indeed in terms of the sort of time base by which evolutionary processes are commonly measured, the time of our overcrowding is right upon us. In less than two centuries we may expect a degree of pressure of population upon means of subsistence which will make the everyday life of everybody a different thing entirely from the prodigally wasteful business it now is.

The basis of this time prediction is found in certain mathematical studies which have been in progress in the writer's laboratory (1) during the past two years. Only a small part of our results have yet been published, but I shall here anticipate some of the as yet unpublished material. Pearl and Reed pointed out that the following conditions should be fulfilled by any equation which is to describe adequately the growth of population in an area of fixed limits.

1. Asymptotic to a line $y = k$ when $x = +\infty$.
2. Asymptotic to a line $y = 0$ when $x = -\infty$.
3. A point of inflection at some point $x = \alpha$ and $y = \beta$.
4. Concave upward to the left of $x = \alpha$ and concave downward to the right of $x = \alpha$.
5. No horizontal slope except at $x = \pm\infty$.
6. Values of y varying continuously from 0 to k as x varies from $-\infty$ to $+\infty$.

In these expressions y denotes population, and x denotes time.

An equation (2) which fulfills these requirements is

$$y = \frac{be^{ax}}{1 + ce^{ax}}, \tag{i}$$

when a, b and c have positive values.

In this equation the following relations hold:

$$x = +\infty, \qquad y = b/c, \tag{ii}$$
$$x = -\infty, \qquad y = 0. \tag{iii}$$

Relations (ii) and (iii) define the asymptotes. The point of inflection is given by $1 - ce^{ax} = 0$, or

$$x = -1/a \log c, \qquad y = b/2c. \tag{iv}$$

The slope at the point of inflection is $ab/4c$.

The general characteristics of the curve given by equation (i) are shown graphically in Fig. 1.

It was recognized that equation (i) gives but a first approximation to one special case of a general law of population growth. We have since developed the mathematical theory much further, and have arrived at a completely general equation, which accounts adequately for cyclical periodicity in population growth, asymmetry of point of

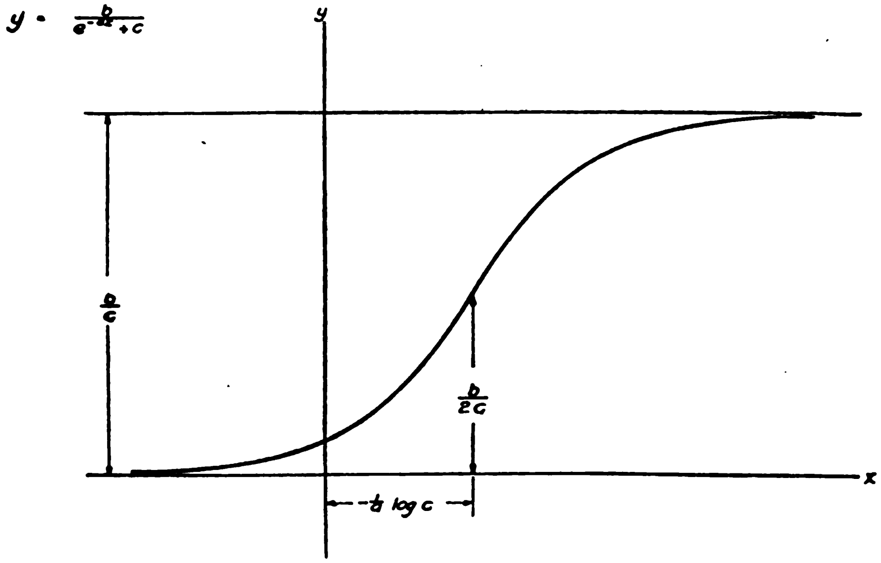

FIG. 1. General form of population growth curves given by equation (i).

inflection, periods of declining population, etc. This will be published shortly, it is hoped.

The more intensive researches have shown, however, that for countries showing normal growth within a single cultural epoch equation (i) is not greatly to be improved upon. The closeness with which it describes the known facts for the United States is shown in Table I, and graphically in Fig. 2.

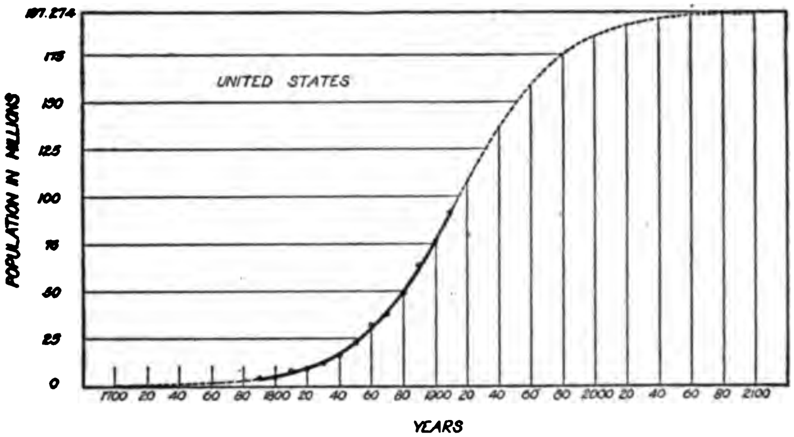

FIG. 2. The growth of the population of the United States.

TABLE I.

Results of fitting population data of the United States, 1790 to 1910, by equation (I).

Year.	Observed population.	Calculated population by equation.	Error.
1790.........	3,929,000	3,929,000	0
1800.........	5,308,000	5,336,000	+ 28,000
1810.........	7,240,000	7,228,000	− 12,000
1820.........	9,638,000	9,757,000	+ 119,000
1830.........	12,866,000	13,109,000	+ 243,000
1840.........	17,069,000	17,506,000	+ 437,000
1850.........	23,192,000	23,192,000	0
1860.........	31,443,000	30,412,000	−1,031,000
1870.........	38,558,000	39,372,000	+ 814,000
1880.........	50,156,000	50,177,000	+ 21,000
1890.........	62,948,000	62,769,000	− 179,000
1900.........	75,995,000	76,870,000	+ 875,000
1910.........	91,972,000	91,972,000	0

The heavy portion of the curve covers the region of known facts given by the census counts indicated on the diagram as small black circles. The light dotted portion of the curve indicates the probable future population growth.

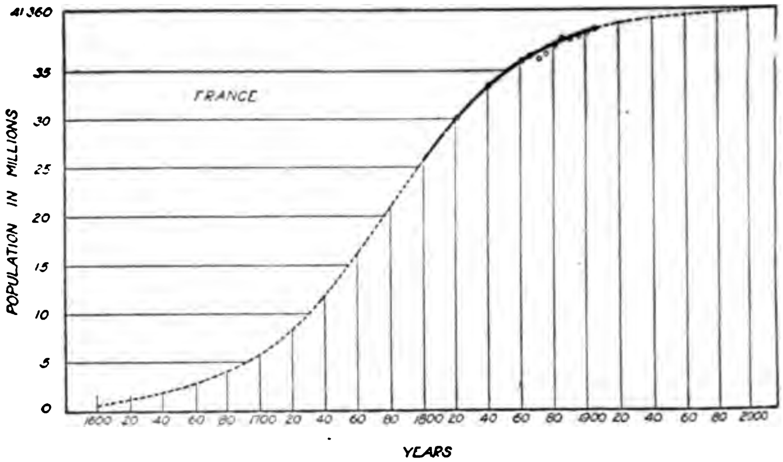

FIG. 3. The growth of the population of France. Significance of lines as in Fig. 2.

The closeness with which the curve fits the known facts, coupled with the fact that it substantially fulfils the *a priori* necessary conditions for a true organic law of population growth, gives confidence in its power to predict future events. In unpublished work we have shown that curves essentially like equation (i) describe with virtually perfect precision the known facts of the population growth for practically all civilized countries for which any census data exists. As further examples Figs. 3 and 4 are inserted which show that the data on population growth in France and in Serbia are described by this curve just as well as are those of the United States.

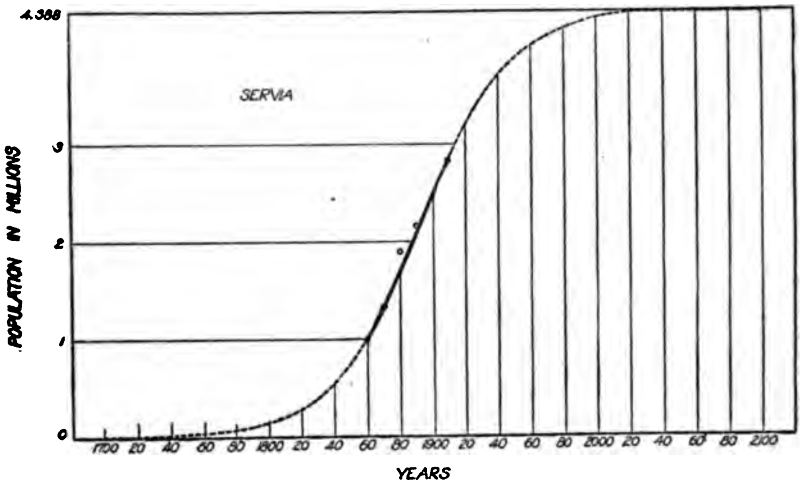

FIG. 4. The growth of the population of Serbia.

Returning to the consideration of the United States we see that, so far as we may rely upon present numerical values, the United States has already passed its period of most rapid population growth, unless there comes into play some factor not now known and which has never operated during the past history of the country to make the rate of growth more rapid. This latter contingency is improbable. The population at the point of inflection works out to have been 98,637,000, which was in fact about the population of the country in 1914.

The upper asymptote given by (i) has the value 197,274,000 roughly. This means that according to equation (i) the maximum population which continental United States, as now areally limited, will have will be roughly twice the present population. This will seem to some a small estimate. It is so easy, and most writers on population have been so prone, to extrapolate population by a geometric series, or by a parabola or some such purely empirical curve, and arrive at stupendous figures, that calm consideration of real probabilities is most difficult to obtain.

Now 197,000,000 people will require, on the basis of our present food habits, about 260,000,000,000,000 calories per annum. The United States during the seven years 1911–1918 produced as an annual average, in the form of human food, *both primary and secondary* (i.e., broadly vegetable and animal), only 137,163,606,000,000 calories per year (3). So that unless our food habits radically change and a man is able to do with less than 3000 to 3500 calories per day, or unless our agricultural production radically increases, it will be necessary when our modest figure for the asymptotic population is reached, to import nearly or quite one half of the calories needed by that population. It seems improbable that the population will go on increasing at any very rapid rate after such a condition is reached.

East (4) has lately made a critical examination of the agricultural limitations to the population of the United States, and concludes "that the agricultural possibilities in this country are not so great as they are generally supposed to be, and . . . even if these possibilities were fulfilled wholly the population the country could support is not enormously greater than the figures Pearl and Reed have set."

Fig. 2 also shows that we shall be uncomfortably close to the asymptotic population by 2100, a time at which the great-grandchildren of a number of persons now living will be components of the population.

What *kind* of a population will that be? What elements now in the population will then, and in the succeeding years, be the dominant ones? Or, will none of the racial groups now so distinct in our population exist as such then, having in the meantime fused and amalgamated, one with the other, till there remains only a biologically homogeneous whole? No one can answer these questions positively today. They are of the future. But we can examine present *tendencies* and past experience. It is to that task that this discussion is addressed. Many others have, of course, written on these problems. Notable papers were those of Mayo-Smith (5). Others who have written along similar lines are Michaud and Giddings (19), Giddings (20), Fishberg (21), Ripley (22), and Alleman (23). But since no recent writer has, so far as I am aware, approached the problem from at all the same point of view as that of the present study, no attempt will be made to review the literature systematically. The point of view throughout this paper is the formulation of the essential problems on which investigation should be, and profitably could be made, rather than any attempt to solve problems. In such formulations there will, of necessity, be clearly pointed out the appalling gaps in our official statistics. Many, indeed most, of the problems to be discussed can only be solved when there is a really adequate system of national bookkeeping, in respect of human life. In pointing out *lacunae* in our national biostatistics the animating motive will be wholly constructive and hopeful of future improvement, and not at all the contrary in respect of either persons or institutions. In general no one is more desirous than the officials charged with the collection and compilation of our national statistics that these statistics should be adequate, in the fullest sense of the word.

II.

Criteria of Biological Americanization.

In a strict sense there are but three general criteria by which the purely biological attributes of a population may be estimated or measured. These are:

1. Somatic physical.
2. Somatic psychological.
3. Biostatistical.

Logically, in a broad sense, all of these may be subsumed under the general title anthropological, but nothing particular is gained by so doing.

In the first category, somatic physical, will be included all those physical attributes of the individuals composing the population, which characterize them as animals, such as hair color, eye color, cephalic index, rate of growth, and so on, right away through the whole gamut of topics commonly designated physical anthropology.

In the second category will be included such attributes as intelligence, habits, feelings, emotions, and such other psychological matters as are capable of definition and measurement with greater or less precision, and at the same time exhibit racial variations of some degree of constancy and specificity.

With neither of these first two criteria shall we deal here, because they have been fully treated by specialists in these particular fields.

The biostatistical criteria form the subject matter of the present discussion. It will be well to examine their scope with some particularity before entering upon detailed treatment of the data.

From the point of view of the population problem, there are two biological phenomena of fundamental importance, *reproduction* and *death*. To study changes in population, of any sort whatever, we need comprehensive and accurate data about each separate element of both of these phenomena. To study progressive or retrogressive secular changes in population the most useful datum we can have is one which combines within itself the essential variations of both reproduction and death. Such a figure I have called (6) the "vital index" of a population.

We have then the following scheme of essential biostatistical criteria of changes in a people.

Statistics of

- A. *Reproduction.*
  - 1. Marriages.
  - 2. Divorces.
  - 3. Births.
    - *a.* Living.
    - *b.* Still.
    - *c.* Illegitimate.
- B. *Death.*
  - 4. Deaths.
- C. *Vital Index.*
  - 5. Birth/Death ratio.

Each of the five groups of this scheme have, of course, many subsidiary elements, some of which we shall be able to consider.

There is one general defect of American vital statistics which so constantly will make itself felt in all the discussion which is to follow that it will be well to say what needs to be said about it, once and for all, at the start. The only index of race stock which we have anywhere in our statistics is such as may be inferred from facts as to the nation in which birth occurred. From the point of view of anything approaching exact science this is a most serious defect.

In the first place, in a number of places besides the United States, the population making up the nation includes a number of perfectly distinct racial stocks. Thus a Canadian, in our vital statistics, is just a Canadian, meaning that he was born in the Dominion of Canada, a political entity. In fact he may be an Indian, a Frenchman, an Englishman, an Eskimo, or of any one of a number of other ethnically distinct subdivisions of the human race.

In the second place, there is one highly important racial group, about which we are legally prevented from getting any biostatistical information. I refer to the Jews. Here, owing to the fact that what is by all odds the biologically purest of all civilized races happens to be also homogeneous in respect of religion, we apparently can have no statistics about it in this country. Whenever an attempt is made, as has repeatedly been the case, to get legislation which will permit the separate statistical enumeration of the Jews, it is blocked by the contention, on the part of some distinguished leaders of the race, that Jewry is a religion and nothing but a religion and does not connote a race. On the other hand others contend that the Jews constitute a highly homogeneous race. By all standards of common sense and anthropology the latter is of course the fact. But leaving quite aside all discussion of verbal definitions about race and religion, the plain fact cannot be successfully controverted that the Jews constitute a highly homogeneous group of people in the American commonwealth. It is for this reason and this alone, I take it, that every competent vital statistician agrees as to the great desirability of having statistical information about them. It is greatly to be hoped that in the not distant future this may be accomplished. So far as an entirely dispassionate and unprejudiced observer can see the Jews themselves could lose nothing, and would gain much if this reform could be established.

In the third place no attempt is made in our vital statistics to follow race stocks, even by nation of birth, for more than one generation. In the case of birth statistics we have the birthplace of the propositus and his parents. In the case of death statistics, we have the same information. In the latter case the information obviously extends further back in point of absolute time, but not in point of generations.

The vital statistician in this country has, perforce, to get into an attitude of mind in which he is grateful for what favors are accorded him. Serious as are the *lacunae* which have been mentioned, it is still possible to draw some reasonably accurate conclusions about race stocks in this country. In other words the case is not quite so bad as it might be. The following statement from "Birth Statistics for the Birth Registration Area of the United States, 1918" (p. 11), "Except for parents born in the United States, however, the country of birth undoubtedly does indicate usually the race stock," unquestionably represents a fact.

We may now turn to the more detailed consideration of the several biostatistical criteria of the changes of populations.

III.

MARRIAGE AND DIVORCE.

Under our existing conditions of law and custom marriage is a substantially essential prerequisite and concomitant of *normal* human reproduction. This does not mean, of course, that spermatozoa and ova will not successfully unite without benefit of clergy. Too often, alas, they do. But anyone who supposes that *normal* human reproduction goes on in our civilization outside of the marital state should examine the statistics of abortions, stillbirths, congenital malformations, and earliest infant mortality, in the cases respectively of legitimate and illegitimate unions. He will find the conditions in the case of illegitimate unions far indeed from biological normality. Consequently it is essential in the statistical discussion of reproduction to take such a factor into account.

There is furthermore in the present connection an especial reason for discussing marriage statistics. Vast quantities of ink have been spilled in the discussion of the " melting pot " in America. It would be unbelievable if it were not true, in view of the interest in this subject, and its obvious importance as a factor in national well-being, that no data are available by which the degree or extent of the " melting " or racial fusion can be measured on a national scale. What makes the case more curious is the fact that the initial element in the fusion is so easily and directly capable of measurement. The first step in racial fusion in this, as any other country, is obviously inter-racial marriage. But there are in this country absolutely no statistics of national scope about the frequency and character of marriages. In a few states, notably New York and Massachusetts, there is official registration (and compilation) of marriage statistics, by much the same mechanism that registers births and deaths. Drachsler (7) has analyzed the New York marriage figures from the point of view of race amalgamation in a notable memoir. But these are exceptional conditions. To know the extent to which the " melting pot " is actually melting we need as the first datum accurate, uniform and complete federal statistics of marriage on an annual basis, and with racial data included.

As these do not exist now, and there is no immediate hope of getting them, we must resort in the present discussion to indirect methods of learning something of the degree to which racial fusion is occurring at the present time. Happily excellent data are at hand for doing this.

Let us begin by defining *an effective marriage within any specified time duration—say one year—as a marriage which produces living offspring within that time period.* Such marriage unions are reproductively effective within that time. This is, of course, the really important consideration. It could possibly make no effect for good or ill to the country if there were a vast number of say Chinese-American marriages, provided all such unions were reproductively absolutely sterile. They would be biologically non-effective marriages, and would not connote the slightest degree of racial fusion. As has already been pointed out, marriage is the first step in racial fusion, but it is *only* the first step. If the marriage does not become reproductively effective there is no true fusion of the races of the contracting parties. So then what, after all, is needed, in addition to straight marriage statistics, is information as to the racial composition of those marriages which at any particular time are *reproductively effective.*

Fortunately data of this latter sort have been regularly published since 1915 for the Birth Registration Area of the United States, collected, checked and compiled by the Division of Vital Statistics of the Bureau of the Census, under the competent direction and supervision of Dr. William H. Davis. Before going into the details as to the character of these statistics it will be well to understand just what is meant by the Birth Registration Area, in order that anyone may judge just what significance is to be attached to its figures, as a sample representing the entire country. In 1919, the year with which we shall deal in the immediately following discussion, the Birth Registration

TABLE II.

Area and population of United States Birth Registration Area in 1919.

United States.		Birth Registration Area.			
		Population.		Land area.	
Population.	Land area (square miles).	Numbers.	Per cent. of total.	Square miles.	Per cent. of total.
104,976,970	2,973,890	61,474,111	58.6	1,075,506	36.2

Area had the geographic and demographic characteristics set forth in Table II. This table is modified from Birth Statistics, 1919, p. 7.

The states included in the Birth Registration Area in 1919 are shown graphically in Fig. 5, which is taken from the frontispiece of Birth Statistics, 1919.

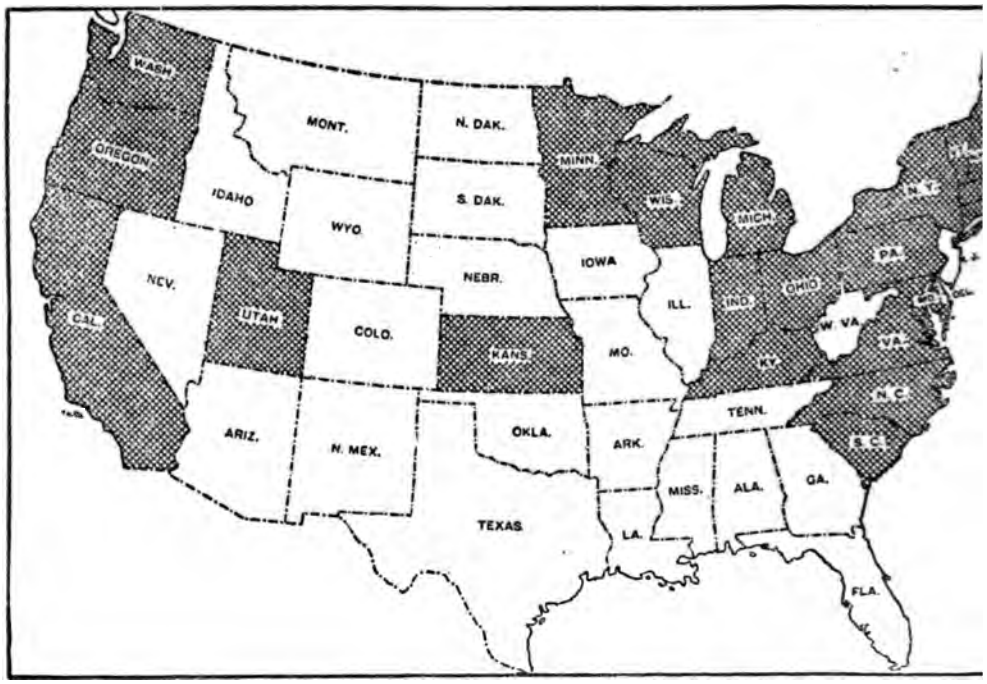
BIRTH REGISTRATION AREA: 1919.

Fig. 5. Map showing states (shaded) in the Birth Registration Area in 1919.

From these data we see that the area in 1919 included nearly six tenths of the entire population of the country, and covered nearly four tenths of the total land area. The Atlantic and Pacific seaboards are practically completely covered, the Middle West is fairly well represented, while the South, the Northwest and the Rocky Mountain regions are practically not represented at all. Probably the most serious omission, from the standpoint of random sampling, is the South. With the reservation which this latter omission implies, we may regard the Birth Registration Area, as constituted in 1919, as fairly representative of the country at large.

TABLE III.

Births (exclusive of stillbirths) of white children, and the country of birth of mother by country of birth of father, in the Registration Area: 1919.

Country of birth of mother.	Country of birth of father.												Totals.
	United States.	Austria (includes Austrian Poland).	Hungary.	Canada.	Denmark, Norway and Sweden.	England, Scotland and Wales.	Ireland.	Germany (includes German Poland).	Italy.	Poland (not specified).	Russia (includes Russian Poland).	Other foreign countries.	
United States	816,546	7,038	1,271	13,033	7,871	8,326	3,788	10,933	11,394	3,634	7,903	8,751	900,488
Austria (includes Austrian Poland)	2,562	46,407	362	34	43	62	22	688	258	615	4,121	599	55,773
Hungary	558	582	14,624	5	8	22	5	307	78	38	313	169	16,709
Canada	11,148	45	7	9,616	111	565	216	121	123	17	65	201	22,235
Denmark, Norway and Sweden	3,583	25	2	110	9,519	140	45	146	32	3	32	222	13,859
England, Scotland and Wales	6,013	92	14	436	143	5,444	459	165	129	23	377	259	13,554
Ireland	5,218	54	4	336	117	702	12,035	140	128	21	29	194	18,978
Germany (includes German Poland)	5,587	465	90	99	132	144	47	6,595	99	216	505	336	14,315
Italy	1,165	65	6	8	8	14	11	11	76,251	8	15	201	77,763
Poland (not specified)	887	274	32	16	11	5	9	97	60	22,562	587	103	24,643
Russia (includes Russian Poland)	2,616	1,670	140	21	25	183	7	310	105	297	53,128	675	59,177
Other foreign countries	3,483	347	73	114	375	204	44	302	503	58	772	30,079	36,354
Totals	859,366	57,064	16,625	23,828	18,368	15,811	16,688	19,815	89,160	27,492	67,847	41,789	1,253,848

In each volume of the Birth Statistics issued by the Bureau of the Census is given a table which shows for each child born the birth nationality, in rather wide groups, of both the father and the mother. This table for 1919 is reproduced here in modified form as Table III. The modifications introduced are: (*a*) male and female births, which are given separately in the original, are here combined, since we are not interested in the sex of the offspring, but only in total marriages effective in 1919; (*b*) the columns and rows of the original table headed respectively "Total foreign," and "Country not stated" are omitted here, as of no purpose in the present inquiry. The net result is that Table III gives all the definitely known facts regarding birth nationality of the partners *in marriages reproductively effective* in 1919 in the Birth Registration Area.

Table III furnishes a picture of the degree of racial assortative mating which was inherent in the marriages which were reproductively effective in 1919 in the Birth Registration Area, or in other words of the degree to which the "melting pot" was melting in that particular year. The data will repay careful and detailed study. One notes at once that the largest frequencies are in those cells which lie on the diagonal of the table connecting the upper left and the lower right corners. These are the cells which exhibit the frequency of effective legitimate matings of like with like in respect of racial stock (in so far as race stock is indicated by nation of birth). Thus there were 816,546 effective marriages in which both male and female were born in the United States: 46,407 in which both members of the pair were born in Austria, and so on. All other cells of the table except those falling on this diagonal theoretically represent racial mixture or fusion of some sort or other. Actually, as we shall presently see, this theoretical assumption is not entirely justified for all the cells. But neglecting this point for the moment in order first to get a broad view, it is plain enough that any coefficient of assortative mating calculated from the table is bound to give a high value. Persons of the same race tend to mate with each other far more than they tend to go outside the race for a mate. The total number of effective marriages included in the table—over one and a quarter millions—is large enough to be impressive.

The next step obviously is to supplement the general impression derived from mere inspection of the table that there is a high degree of racial associative mating (like mating with like), with a more exact determination of its magnitude. The method which at once occurs to one to do this is to calculate by the contingency method the coefficient of racial assortative mating. This I have not done in the present instance for two reasons: first because I know about what the value would be from experience with other tables of racial assortative mating which I have dealt with by the contingency method before. Without correction of non-diagonal, but really like × like cells, the coefficient of contingency will be somewhere in the neighborhood of $+0.8$. The second, and more important reason, is that the coefficient in reality tells very little more than common-sense inspection of the table tells, being in fact merely a single numerical expression of that common-sense result, and gives nothing like so much useful information as does the method of analysis of the material upon which we shall presently embark.

Before doing this I wish to discuss at the outstart one source of error implicit in the table which will be neglected throughout, because its magnitude is so small that it cannot sensibly affect any result, whereas to correct for it would require an inordinate expenditure of time and labor, even if, with the available data, it ever could be fully corrected. The error is this. I have referred throughout to the frequencies of Table III as frequencies of "effective marriages." Actually, of course, they are frequencies of births. Not all births are single births. Some are twins, triplets, etc. In the case of twins the *same* marriage gets counted in Table III as effective twice in 1919. So, of course, it was in fact from the standpoint of population growth. But from the standpoint of analyzing the racial fusions involved it apparently implies one more pair of parents of the particular sort of racial combinations involved than in fact existed. The number of multiple births, however, in proportion to total births, is so small as to make this a negligible source of error in any conclusions which will here be drawn from the figures. Thus in 1919 there were 12.2 cases of plural births per 1000 mothers of that year. Or, put in another way, there were only 32,204 live infants born plurally, in a total of 1,373,438 live infants born in 1919.

The first problem on which interest centers may be stated in this way: what proportion of the marriages effective in adding to the population in 1919, were racially pure (race as always being indicated by country of birth), as compared with those which were racially mixed, the mixing being either (*a*) of American-born with foreign-born, or (*b*) of various different sorts of foreign-born together?

To get a first approximation to the answer to this question Table IV has been prepared from Table III and similar data in Birth Statistics, 1919, showing the results for the Registration Area as a whole and for each state separately. The columns contain the following data:

Column A.—The sum of the diagonal cells of Table III (and similar tables for each state). The entries for this column show the total number of births from effective marriages in which both members of the pair were born *in the same country,* and were thus inferentially of the same racial stock. As has already been pointed out this inference is, in a broad way, justified, except for the United States. There we step over into another "universe of discourse." Even there the two members of the married pair are at least alike in these respects, that: (1) they were both born in the United States, and (2) they have been subjected to Americanizing influences for an average period of from 28 to 33* years. This means that they went, in general, to American schools and were subjected to Americanizing influences throughout the most formative period of life. Although real racial differences are not to be wiped out in any such easy way or short period as this, still no one who has attentively observed our foreign peoples will deny that the individual born and reared in this country is in many respects definitely different from the foreign-born immigrant, coming at any age after say 12 to 15. For the present then, we shall treat the U. S. × U. S. effective marriages as though they were directly comparable to the other diagonal cell groups; reverting later to further discussion of the point.

Column B.—This column contains the sum of the frequencies in the cells forming the first *row* of Table III, *exclusive* of the first cell in the row (U. S. × U. S.). The figure thus shows how many effective marriages in 1919 there were in the B. R. A.,* in which the *female* member of the pair was American born. This will, in general, indicate the extent of the assimilation of foreign males by the American females of the existing population.

Column C.—This contains the sum of the frequencies in the cells forming the first *column* of Table III, again exclusive of the topmost cell (U. S. × U. S.). This is the counterpart of Column *B,* showing the extent of effective fusion of American-born male elements with foreign-born females. Columns *B* and *C* together show the whole extent of the effective fusion in the B. R. A. in 1919 of foreign with native-born elements.

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* These figures are respectively about the average ages of mothers and fathers in 1919.

* This will be used as a convenient abbreviation of Birth Registration Area throughout the remainder of the discussion.

Column D gives the sums of the figures in Columns *B* and *C* for the purpose above indicated.

Column E gives the total number of effective marriages in which both partners were born in the United States. This is the frequency in the upper left-hand corner cell of Table III, and the corresponding cell for the state tables.

Column F gives the total frequency in all the diagonal cells of Table III, *exclusive* of the corner cells dealt with in Column *E*. In other words these figures show the total number of racially *pure* effective marriages in which both partners were foreign-born.

Column G contains the sum of the frequencies in all those cells of Table III which *are not included* in Columns *A, B,* and *C* of Table IV. These are the cells which fall neither upon the diagonal, nor upon the bordering row or column of the table. The summed frequencies represent the total number of effective marriages in which racial mixture or fusion occurred, with neither partner in the union American-born. The figures show the extent to which the several foreign elements in our population are failing to keep racially pure, without at the same time mixing with American-born.

Column H gives the sums of the entries in Columns *F* and *G*. These figures show the total numbers of effective marriages in the B. R. A. in 1919 in which both members of the united pair were of foreign birth.

Column I gives the percentage which each entry in Column *D* is of the corresponding entry in Column *A*. In other words the figures in Column *I* answer the following question: what proportion do the effective marriages which represent a fusion or "melting" of foreign stocks *with American-born elements* form of the effective marriages in which like mated with like racially (counting U. S. × U. S. as a like mating)?

Column J gives the percentages which the several entries in Column *D* are of the corresponding entries in Column *E*. These figures show the proportions which effective fusions of native-born with foreign-born elements form of effective matings of native-born with native-born. This column may be taken to indicate the rate at which true net amalgamation is taking place.

Column K gives the percentages which the several entries i Column *D* are of the corresponding entries in Column *F*. The figure show the relative tendencies of foreign stocks to mate (effectively with native-born stocks, as compared with their tendency to mat (effectively) within their own racial groups in this country.

Column L gives the percentage which each entry in Column *F* i of the corresponding entry in Column *E*. The figures show the proportions of racially pure effective matings of foreign-born as compare with native-born elements.

Column M gives the percentage which each entry in Column *G* i of the corresponding entry in Column *F*. The figures indicate th relative tendency of the foreign-born stocks to mate (among themselves) pure, as compared with their tendency to mate with othe foreign-born stocks.

Column N gives the percentage which each entry in Column *H* is o the corresponding entry in Column *E*. The figures compare relativel the effective results of matings in which both parties are foreign-bor with those matings in which both partners are native-born.

The several states in Table IV are arranged in descending orde of the entries in Column *I*.

In the discussion of Table IV it will be well to confine the attentio in the first instance to the percentage columns beginning with *I*, noting only that the total number of effective marriages dealt with, over a million, is large enough to command respect. From the table the following points are especially to be noted.

1. For the entire B. R. A. the percentage of amalgamation or fusion of foreign-born stocks with native-born, as compared with racially like effective matings of all sorts, as indicated by effective marriages in 1919, is just under 11.5 per cent. That is, for about every ten effective marriages in which the partners were of like nativity there was one in which one partner was of American and the other of foreign nativity. On the whole this appears a reasonably large proportion, when one considers inherent prejudices which must be overcome before such marriages can occur. Of course one realizes fully that some unknown, but certainly significant proportion of the 126,762 American × foreign-born cross matings are really not *racially* cross at all but like, one partner merely having been born in the United States. But fully granting this, there are two further points to be noted, namely: first, that American birth and upbringing to marrying age tend to create a considerable measure of antipathy or

TABLE IV

Showing for the B. R. A., and each separate state certain relations of effective marriages in 1919.

Area.	*A* All like × like matings.	*B* Native ♀♀ × foreign ♂♂.	*C* Native ♂♂ × foreign ♀♀.	*D* Sum of *B* and *C*.	*E* Native ♂♂ × native ♀♀.	*F* All foreign pure matings.	*G* All foreign mixed matings.	*H* Sum of *F* and *G*.	*I* Per cent. which *D* is of *A*.	*J* Per cent. which *D* is of *E*.	*K* Per cent. which *D* is of *F*.	*L* Per cent. which *F* is of *E*.	*M* Per cent. which *G* is of *F*.	*N* Per cent. which *H* is of *E*.
Total B. R. A.	1,102,806	83,942	42,820	126,762	816,546	286,260	24,280	310,540	11.49	15.52	44.28	35.06	8.48	38.03
New Hampshire	6,689	996	788	1,784	4,317	2,372	129	2,501	26.67	41.32	75.21	54.95	5.44	57.93
Maine	12,196	1,545	1,448	2,993	9,888	2,308	109	2,417	24.54	30.27	129.68	23.34	4.72	24.44
Massachusetts	67,190	8,217	6,915	15,132	31,468	35,722	2,437	38,159	22.52	48.09	42.36	113.52	6.82	121.26
Minnesota	40,920	6,566	2,625	9,191	33,028	7,892	705	8,597	22.46	27.83	116.46	23.89	8.93	26.03
Washington	19,416	2,455	1,244	3,699	16,412	3,004	458	3,462	19.05	22.54	123.14	18.30	15.25	21.09
Michigan	68,161	7,909	4,080	11,989	50,114	18,047	1,913	19,960	17.59	23.92	66.43	36.01	10.60	39.83
California	42,293	4,888	2,169	7,057	31,333	10,960	1,039	11,999	16.69	22.52	64.39	34.98	9.48	38.30
Wisconsin	45,692	5,277	2,118	7,395	38,329	7,363	678	8,041	16.18	19.29	100.43	19.21	9.21	20.98
Vermont	5,910	551	401	952	4,923	987	63	1,050	16.11	19.34	96.45	20.05	6.38	21.33
Connecticut	27,638	2,663	1,566	4,229	11,468	16,170	1,151	17,321	15.30	36.88	26.15	141.00	7.12	151.04
New York	183,463	17,983	9,202	27,185	93,947	89,516	9,578	99,094	14.82	28.93	30.37	95.28	10.70	105.48
Oregon	11,290	994	513	1,507	10,284	1,006	192	1,198	13.35	14.65	149.80	9.78	19.09	11.65
Utah	11,216	810	611	1,421	10,304	912	103	1,015	12.67	13.79	155.81	8.85	11.29	9.85
Pennsylvania	176,840	13,029	4,789	17,818	117,171	59,669	3,541	63,210	10.08	15.21	29.86	50.92	5.93	53.95
District of Columbia	5,255	300	174	474	4,736	519	66	585	9.02	10.01	91.32	10.96	12.72	12.35
Ohio	99,556	5,067	2,206	7,273	79,342	20,214	1,305	21,519	7.31	9.17	35.98	25.48	6.46	27.12
Maryland	25,347	1,084	535	1,619	22,579	2,768	302	3,070	6.39	7.17	58.49	12.26	10.91	13.60
Kansas	33,180	1,255	530	1,785	31,493	1,687	97	1,784	5.38	5.67	105.81	5.36	5.75	5.66
Indiana	55,027	1,454	566	2,020	51,000	4,027	283	4,310	3.67	3.96	50.16	7.90	7.03	8.45
Virginia	40,334	395	159	554	39,703	631	70	701	1.37	1.40	87.80	1.59	11.09	1.77
Kentucky	52,820	312	112	424	52,532	288	43	331	.80	.81	147.22	.55	14.93	.63
South Carolina	21,681	84	24	108	21,586	95	10	105	.50	.50	113.68	.44	10.53	.49
North Carolina	50,692	108	45	153	50,589	103	8	111	.30	.30	148.54	.20	7.77	.22

prejudice towards the more recent immigrant
making this statement the writer is relying on
servations of foreign stocks in our country.
American-born child of foreign parents desires
"pure American," to slough off and forget
characterize, as he or she thinks, the "wop," u
term of disparagement for the foreigner. U
names and customs of all sorts, excite in ma
phobic reaction in the native-born children of
How unfortunate this all is is only too often app
substitutes for the dropped foreign habits are
stitutes indeed. The ignorant and foolish child
of American habits and manners, while lettin
distinctly fine and precious.

In the second place it must be remembere
effective marriages of native-born there are man
ings. Perhaps this balances off in good part
cross-mating figures.

2. Thirteen states exhibit a fusion percen
average, with New Hampshire followed by M
approximately one quarter of all the effective mar
and foreign-born partners. In these two states
these mixed marriages includes a French Ca
partners. The group above the average in fusio
all the New England States except Rhode Islan
B. R. A.) and New York. This would be reason
are old states in which (*a*) there has been tim
ficial and frothy aspects of race prejudice to e
foreign population has, by and large, been of rat
quality. In the Middle West, Minnesota, Mic
give percentages well above the average. Here a
born elements have been long in residence an
worth. From the Far West, Washington, Califo
give percentages more or less above the average.
accounted for by the rather distinctly open-mi
of these communities, so long as Orientals are n

3. The distinctive feature of the group of
percentages below the average is that it is out
group. Seven out of the ten states in the g
Mason-Dixon line. This is probably to be a

factors: (*a*) an intense prejudice against racial miscegenation in general, founded upon the concrete case of the negro, and (*b*) a relatively small proportion of foreign-born in the communities concerned.

4. This brings up a question of obvious interest. When the states are arranged in descending order of effective race fusion percentages as in Table IV (from Column *I*), is not this also about the same order in which they would stand in respect of percentage of foreign-born white populations? Table IV shows that this is in fact the case to a high degree of approximation.

TABLE IV *a*.

Comparing percentages of foreign-born whites in the total population in 1910 with Column I of Table IV.

States.	Column *I*. Table IV. fusion percentage.	Per cent. of foreign-born whites in population in 1910.
New Hampshire	26.67	22.4
Maine	24.54	14.8
Massachusetts	22.52	31.2
Minnesota	22.46	26.2
Washington	19.05	21.1
Michigan	17.59	21.2
California	16.69	21.8
Wisconsin	16.18	22.0
Vermont	16.11	14.0
Connecticut	15.30	29.5
New York	14.82	29.9
Oregon	13.35	15.3
Utah	12.67	17.0
Pennsylvania	10.08	18.8
District of Columbia	9.02	7.4
Ohio	7.31	12.5
Maryland	6.39	8.0
Kansas	5.38	8.0
Indiana	3.67	5.9
Virginia	1.37	1.3
Kentucky	.80	1.7
South Carolina	.50	0.4
North Carolina	.30	0.3

How close the parallelism is between the two columns of Table IV *a* is shown graphically in Fig. 6.

The important fact strikingly demonstrated in Table IV*a* and Fig. 6 is that the amount of racial amalgamation or fusion going on

in the several parts of the American " melting
in the most direct and close way to the amount
stock in the local population. In other words th
standing factor in determining whether there s
assimilation of foreign elements into the establish

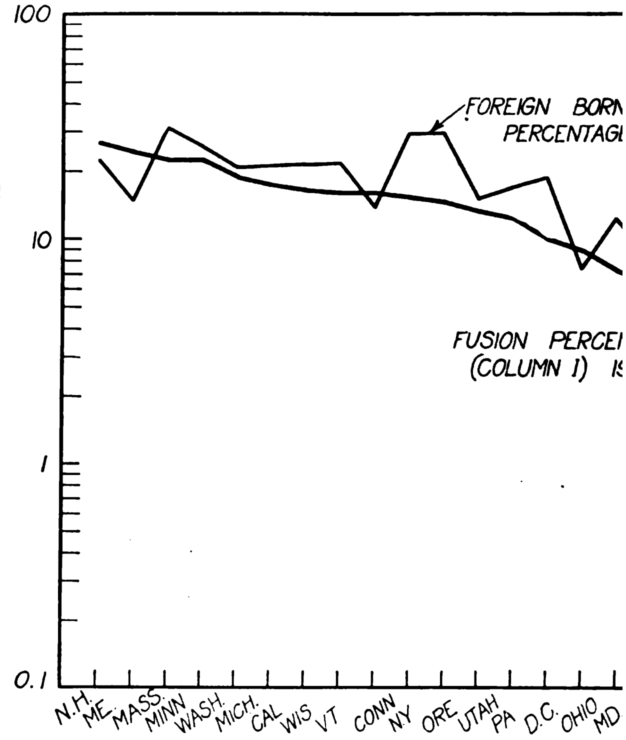

FIG. 6. Showing the close agreement which exists
of mixed marriages of native and foreign-born effective i
ages in the population of foreign-born whites in 1910.

tion is simply the opportunity afforded by pr
statistically one of the most potent factors in bri
all marriages. Given a community in which the
born whites as more or less settled residents, the
many marriages in which one of the partners will
the other of foreign nativity.

5. Turning to Column *J* of Table IV, which
which the total number of reproductively effect

which one partner was American-born, is of the number of effective matings in which both partners are native-born, it is seen that while the percentages are, as must necessarily be the case, somewhat higher than those of Column *I*, they nevertheless follow about the same order. Some of the individual entries are of interest. Thus in the case of Massachusetts there were nearly one half (48.09 per cent.) as many matings effective in producing offspring in 1919 in which fusion of American- and foreign-born occurred, as there were effective matings where both partners were native-born. Just as in the preceding case it is evident that opportunity is the important factor in making the melting pot melt. Figure 7 is of the same general character as Fig. 6 except that here the *J* Column percentages, which may be taken as the closest approximation to actual, net, effective racial amalgamation, are plotted against percentages of foreign-born whites in 1910.

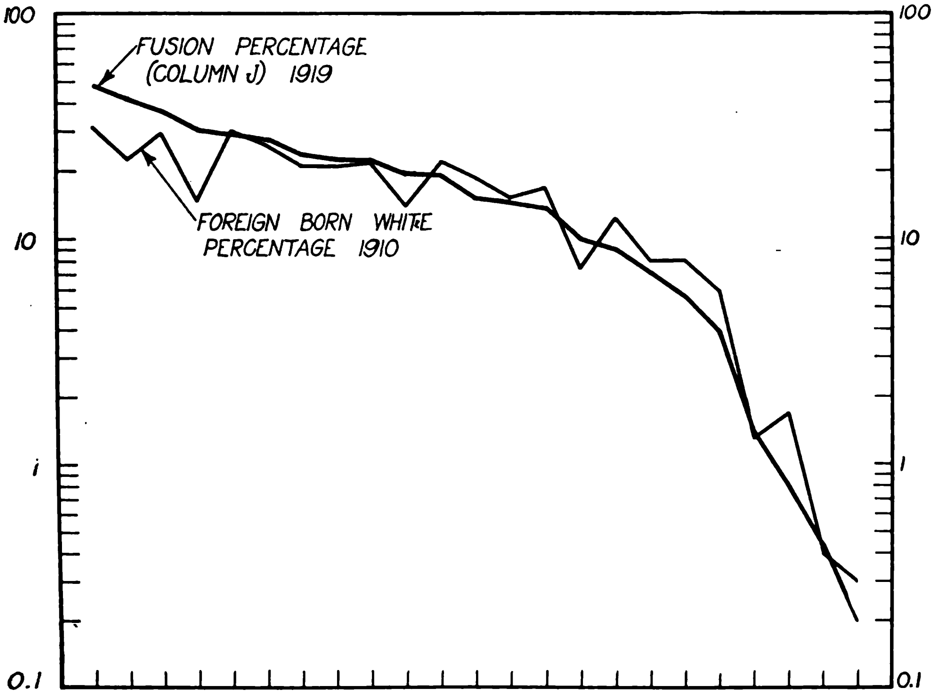

FIG. 7. Showing the agreement between percentages of mixed American and foreign effective marriages in 1919 (percentages on native × native base only), and percentages of foreign-born whites in the population in 1910.

The agreement between the two lines is even closer in this diagram than was the case in Fig. 6. There can be no doubt that where the most foreign-born whites are, there occurs the most mixed mating with native-born, not only absolutely but also relatively, in proportion both to all racially pure matings and to purely American (i.e., native × native) matings.

And there can be no doubt, under the circumstances, about the reality of racial fusion in this country. When from one fourth to one half as many children are produced in a given year from mixed matings (in which one partner is American) as from matings of strictly American-born persons, amalgamation of the immigrant elements into the previously existing complex is certainly going on apace.

6. Column *K* gives us a view of the same phenomenon from another angle. Here the question presented is: what proportion do effective mixed matings (in which one partner is American-born) form of racially pure matings in the same communities in which both partners are of foreign birth? In ten out of the twenty-three states there are *more* effective mixed matings of the amalgamating type—or, if one chooses, of the Americanizing type—than there are of racially pure foreign × foreign matings. These are the states exhibiting percentages greater than 100 in Column *K*. A better appreciation of the essential point here can be gained by a graphic presentation. This is given in Fig. 8, where the data of Column *K* are plotted against the percentages of foreign-born whites in 1910. In the diagram the states are arranged in descending order of proportion of foreign-born whites in 1910 (heavy line). This, it will be noticed, is the opposite of the plan in Figs. 6 and 7, where these data were given as the light instead of the heavy line.

It is at once apparent that, in general, there is no significant association between the two variables here plotted. Leaving out of account for the moment the three states with the highest percentage of foreign-born whites, namely, Massachusetts, New York and Connecticut, it is clear that the remaining 20 states proceed across the diagram in what amounts to a horizontal line, in respect of the proportion of fusion to pure all-foreign matings, while at the same time the percentage of foreign-born in the population is steadily decreasing. In the eastern states mentioned the case is different. The proportion of foreign-born whites in total population is there highest. The proportion of fusion matings to pure foreign-born matings is practically the lowest. Here we see the effect of the condensed, homo-

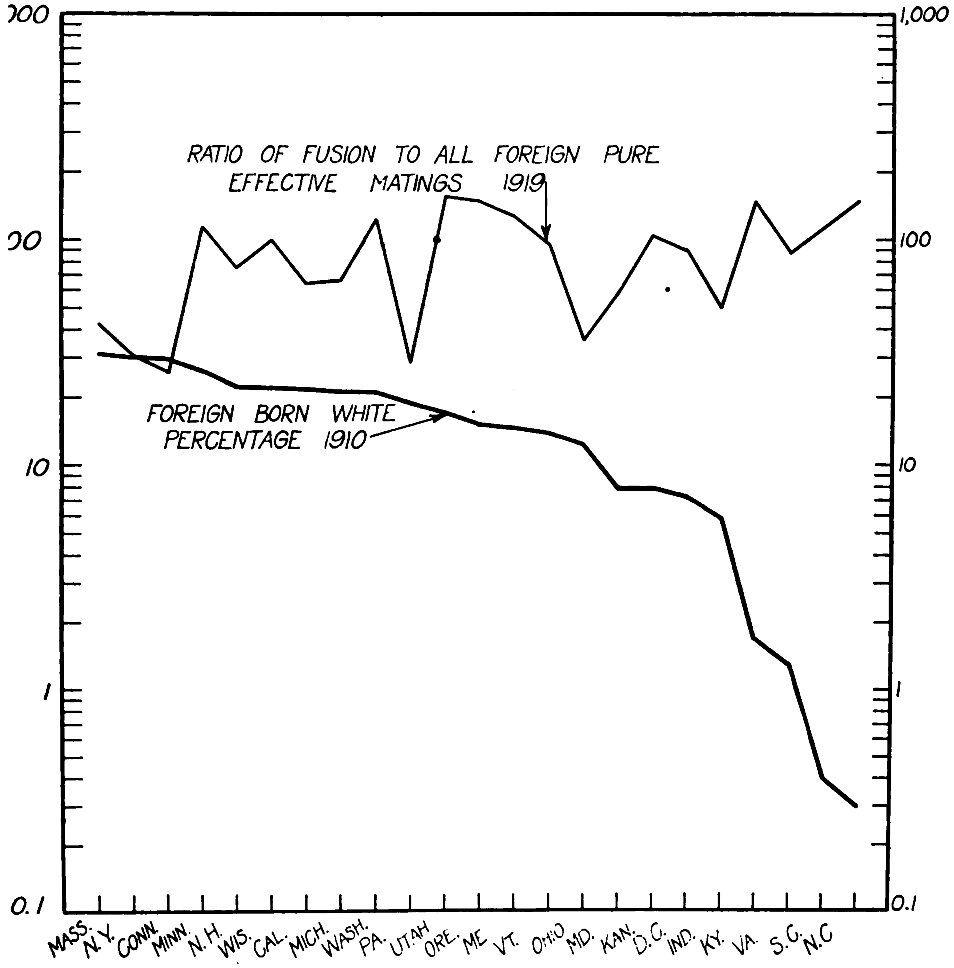

Fig. 8. Showing the relation between (*a*) the proportion of foreign-born whites in the population in 1910, and (*b*) the percentage which "fusion" effective matings (as defined in text) are of effective all-foreign pure matings in the same communities.

geneous "foreign colony" type of population, flourishing in great industrial centers. Yet the fact must not be overlooked that all three of these states, Massachusetts, Connecticut and New York, are above the average in the percentage which fusion matings form of pure American-born matings (cf. Column *J*). There is no contradiction in

these two facts, though at first glance there might facts merely mean that in each of these three comm comparatively large, well-settled, foreign-born pop ing at a relatively much more rapid rate, both pu with American-born stock, than is the strictly A by itself.

7. Column *L* gives for each state the percent foreign-born × foreign-born matings form of the e

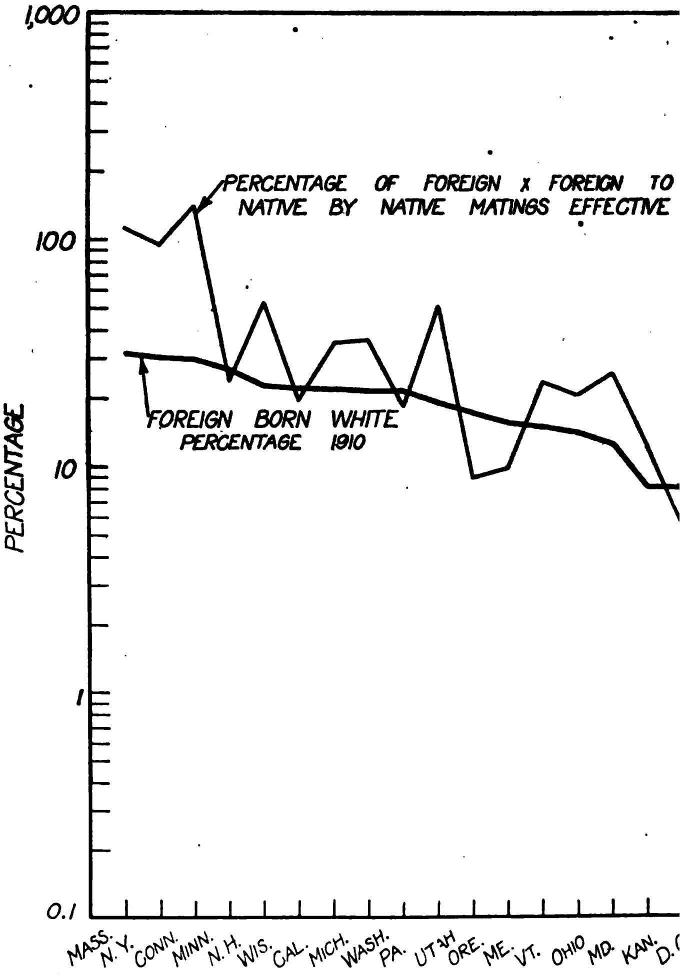

FIG. 9. Showing the relation between (*a*) percentage o population in 1910 (heavy line), and (*b*) proportion of fore to native matings effective in 1919.

× native-born matings. Plotting these results against the percentage of foreign-born in the total population in 1910 gives the result shown in Fig. 9.

It is evident that the two curves run generally together, meaning that the proportion of foreign × foreign effective matings tends to be highest where there are proportionally the greatest number of foreign-born in the population, and lowest where there are the fewest. But there are some striking outstanding points, which merit special consideration. In the three states Massachusetts, New York and Connecticut there were actually, in 1919, as many or more matings of the type foreign-born × foreign-born, as there were of the type native-born × native-born, *effective in producing offspring.* This means, put in another way, that in these three states as large or larger additions to the future population were made in 1919 by the foreign-born as by the native-born. Furthermore in three other states, namely New Hampshire, Pennsylvania and Maine, the effective foreign × foreign matings were more than half as numerous as the effective native × native.

There can be no doubt that, on the average, by whatsoever standard measured, the native-born are "Americanized" in higher degree than the foreign-born. It would appear that in the six states named, the less Americanized elements are multiplying too fast in proportion to the more Americanized. There can furthermore be no doubt that this state of affairs arises in largest degree as a consequence of the crowding of large, purely foreign "colonies" in great industrial centers of population. This is certainly true of Massachusetts, New York, Connecticut and Pennsylvania. In Maine and New Hampshire the same mechanism essentially is at work and is the *causa princeps* of the result, but it operates chiefly on a different sort of foreigner, namely the French-Canadian, about whose eventual and satisfactory Americanization no particular doubt can be felt. In the other four states named, what appears to be needed chiefly is to break up the too close geographical segregation. Then the problem will take care of itself. This appears to me to be abundantly proved by the results which have already been presented here. Give the foreign elements a chance to meet and really know the native population, and the melting pot promptly begins to seethe and bubble. The blame for the sort of conditions depicted by the statistics of Massachusetts, New York, Connecticut and Pennsylvania, in my judgment, lies far heavier upon the native Americans than upon the foreigners. Similarly situated we should all act much as the foreigners do in respect of our mating habits.

8. Column *M* presents another angle of the problem. It shows the percentage which effective all-foreign mixed matings are of effective all-foreign pure matings. The all-foreign mixed matings are those in which both partners are foreign-born, but the husband has a different country of birth than the wife. In other words we have in this column a measure of the extent to which assortative mating in respect of race occurs among the foreign-born in this country, excluding all mixtures of foreign with American-born. It is seen at once that all percentages are relatively small, the largest being 19.09 in the case of Oregon. The comparison between the entries of Column *M* and those of Column *K* is highly significant. The foreigner in this country is much more likely to marry an American-born person, if he does not marry one of his own race, than he is to marry some other foreigner not of his own race. This fact would become even more emphasized if we were able to make the proper corrections for Jews of different countries of birth.

9. Column *N* compares the total of effective matings of foreign × foreign-born (whether pure or mixed) with the total of native × native effective matings. In three states, Massachusetts, New York and Connecticut, these percentages are over 100, as would of course be expected from what has gone before. In these states the foreign-born produced more children in 1919 than did the native-born. But, after all, the *children* were native-born and will be subjected to Americanizing influences from birth onwards.

Up to this point we have been considering very broad types of matings, and examining the extent of effective fusion of races in different parts of the B. R. A. It will now be well to examine the facts from another angle, and from one point of view summarize them.

Table V shows that in 1919 there were in the B. R. A. 78 racially different types of mating effective in producing offspring, not separating sexes of either mated partners or offspring. These matings are given in descending order of number of children produced in Table V. It is to be understood, here as before, of course, that " race combination " merely means " so far as indicated by country of birth," and is in so far and in that sense not accurate.

From Table V it is seen that 65 per cent. of the children produced in the B. R. A. in 1919 were from American-born parents. Of the remaining 35 per cent. the make-up was as follows: 10.113 per cent.

TABLE V.

Net effectiveness in producing next generation.

Order.	Race combination.	Number of births.	Per cent.
1	U. S.×U. S.	816,546	65.123
2	Italy×Italy	76,251	6.081
3	Russia×Russia	53,128	4.237
4	Austria×Austria	46,407	3.701
5	Other foreign×other foreign	30,079	2.399
6	U. S.×Canada	24,181	1.929
7	Poland×Poland	22,562	1.799
8	U. S.×Germany	16,520	1.318
9	Hungary×Hungary	14,624	1.166
10	U. S.×U. K.	14,339	1.144
11	U. S.×Italy	12,559	1.002
12	U. S.×other foreign	12,234	.976
13	Ireland×Ireland	12,035	.960
14	U. S.×Scandinavia	11,454	.914
15	U. S.×Russia	10,519	.839
16	Canada×Canada	9,616	.767
17	U. S.×Austria	9,600	.766
18	Scandinavia×Scandinavia	9,519	.759
19	U. S.×Ireland	9,006	.718
20	Germany×Germany	6,595	.526
21	Austria×Russia	5,791	.462
22	U. K.×U. K.	5,444	.434
23	U. S.×Poland	4,521	.361
24	U. S.×Hungary	1,829	.146
25	Russia×other foreign	1,447	.115
26	U. K.×Ireland	1,161	.093
27	Austria×Germany	1,153	.092
28	Canada×U. K.	1,001	.080
29	Austria×other foreign	946	.075
30	Austria×Hungary	944	.075
31	Austria×Poland	889	.071
32	Poland×Russia	884	.071
33	Germany×Russia	815	.065
34	Italy×other foreign	704	.056
35	Germany×other foreign	638	.051
36	Scandinavia×other foreign	597	.048
37	U. K.×Russia	560	.045
38	Canada×Ireland	552	.044
39	U. K.×other foreign	463	.037
40	Hungary×Russia	453	.036
41	Hungary×Germany	397	.032
42	Austria×Italy	323	.026
43	Canada×other foreign	315	.025
44	Germany×Poland	313	.025
45	U. K.×Germany	309	.025
46	Scandinavia×U. K.	283	.023
47	Scandinavia×Germany	278	.022
48	Hungary×other foreign	242	.019
49	Ireland×other foreign	238	.019
50	Canada×Scandinavia	221	.018
51	Canada×Germany	220	.018
52	Ireland×Germany	187	.015
53	Scandinavia×Ireland	162	.013
54	Poland×other foreign	161	.013
55	Austria×U. K.	154	.012
56	U. K.×Italy	143	.011
57	Ireland×Italy	139	.011

Order.	Race combination.	Number of births.	Per cent.	Order.	Race combination.	Number of births.	Per cent.
58	Canada×Italy	131	.010	69	Scandinavia× Italy	40	.0032
59	Italy×Russia	120	.0096	70	Hungary×U. K.	36	.0029
60	Germany×Italy	110	.0088	71	Ireland×Russia	36	.0029
61	Canada×Russia	86	.0069	72	Canada×Poland	33	.0026
62	Hungary×Italy	84	.0067	73	Ireland×Poland	30	.0024
63	Austria×Canada	79	.0063	74	U. K.×Poland	28	.0022
64	Austria×Ireland	76	.0061	75	Scandinavia× Poland	14	.0011
65	Hungary× Poland	70	.0056	76	Hungary× Canada	12	.00096
66	Austria× Scandinavia	68	.0054	77	Hungary× Scandinavia	10	.0008
67	Italy×Poland	68	.0054				
68	Scandinavia× Russia	57	.0045	78	Hungary× Ireland	9	.0007
					Total	1,253,848	100.004

of the total children had one parent native-born, leaving approximately 25 per cent. of the total births having both parents foreign-born. So then it appears that almost exactly 75 per cent. of the total children had one or both parents native-born. Of the foreign-born effective matings the highest percentage is exhibited by the Italians, next by the Russian born, next by the Austrian born, and next by the residue group "other foreigners." These four foreign groups contributed together some 16 per cent. of the total children. It is probably conservative to estimate that one half at least of these parental groups were Jewish. The first twelve of the matings in the table produced over 90 per cent. of the children born in the B. R. A. in 1919. Whether the above facts, which are shown graphically in Fig. 10, will be regarded as alarming or reassuring depends upon one's preconceived point of view. From one point of view it will be regarded an evil of alarming proportions that but 65 per cent. of children born in this country have both parents native-born. But, on the other hand, as the figures show, the proportion having both parents native-born is overwhelmingly larger than that for any other mating. Our present task, however, is simply to present the facts, to as great a degree of approximation as is possible.

Throughout the discussion of this point we have been compelled, by lack of the raw statistical data necessary to do anything else, to take country of birth as indicative of race. Everyone knows that it is not accurately so. It is at best only an approximation, and conceals

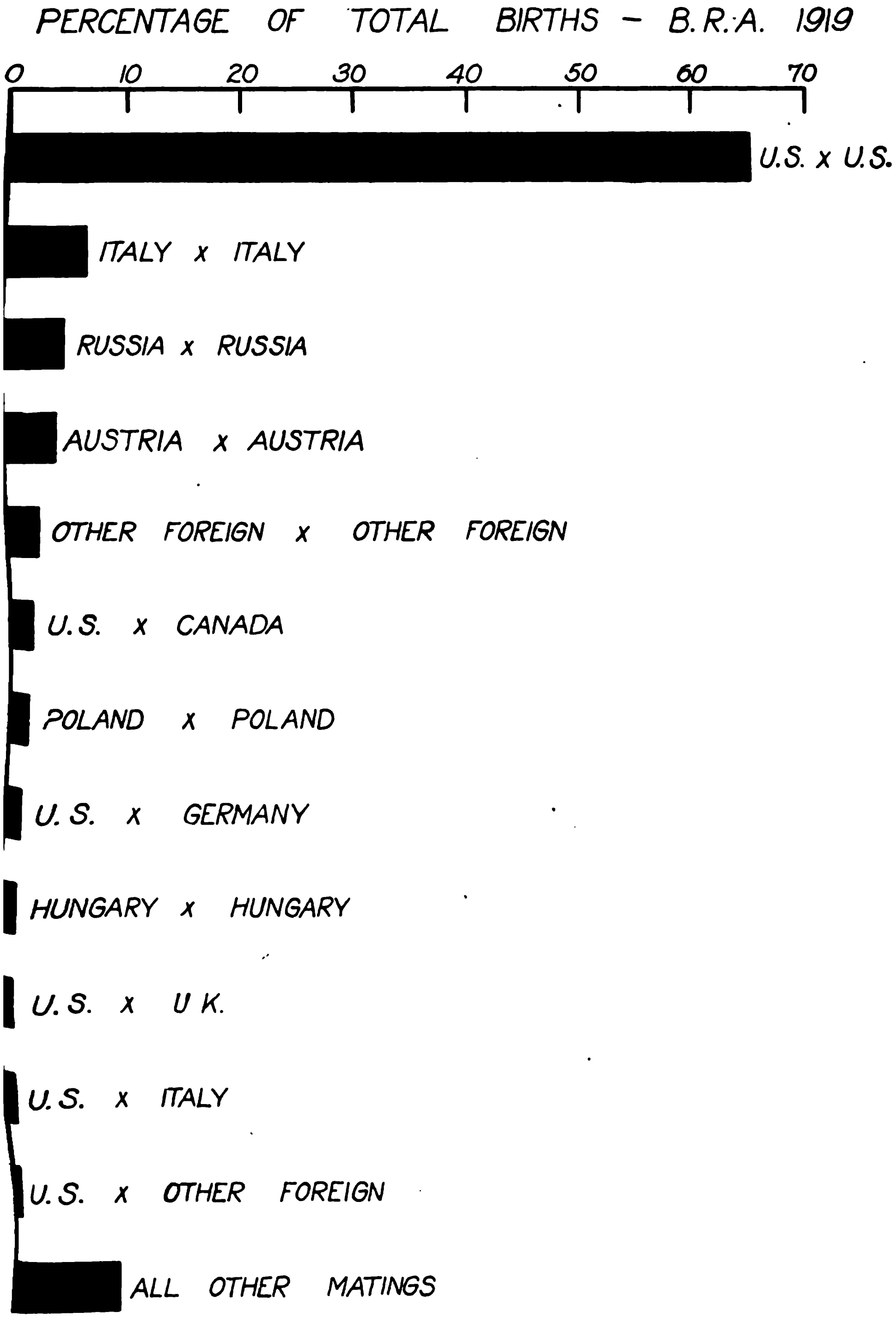

Fig. 10. Bar diagram showing proportion of children produced in the B. R. A. in 1919, from different types of matings.

many pertinent facts. Again, because of the lack of the necessary data, it is impossible to make any correction of Tables III, IV and V which will, after the expenditure of a considerable amount of time that would be involved in attempting corrections, tell us anything more exact than what we know to be the broad features of the case. The errors in Tables III, IV and V incident to taking birthplace as an index of race are, broadly speaking, as follows:

1. An error which probably tends to make the degree of racial assortative mating in effective marriages appear *lower* than it really is, is found in the fact that the U. S. × U. S. group contains many different racial elements. Many of these probably mate assortatively and should, on a strictly racial basis, be taken out of the upper left hand corner cell of Table III and distributed to their proper diagonal cells down the table. But others of this sort do not mate in strict racial assorting, and they should be distributed to the appropriate cells of the table off the diagonal.

2. Another factor of much greater importance in lowering the apparent below the true degree of racial assortative mating, is found in the cells not on the diagonal in Table III with disproportionately high frequencies; as for example, U. S. ♂ × Russian ♀ (frequency 2616), Austrian ♂ × Russian ♀ (frequency 1670), etc. Now while these appear to be cross-matings, as a matter of obvious fact, they are in the vast majority of cases strictly pure matings, namely of Jewish ♂ × Jewish ♀. They should in some unknown, but certainly large proportion, be transferred to the diagonal cells. Another example of the same thing, but not involving Jews, is seen in the disproportionately high frequencies in the cells Canadian ♂ × U. K. ♀ (frequency 436) and U. K. ♂ × Canada ♀ (frequency 565). What we really have, in a great proportion of these cases, is the mating of Canadian-born English, Welsh or Irish males with foreign-born females *of the same race.*

The first of the sources of error named I am personally inclined largely to discount, because of the conviction that about the only logically tenable simple definition of an American, in the sense the word is here used, is that of nativity. An American is a person born in the United States.* The only *racially* pure American is the Amer-ind. Everybody else is something other than American racially. I can not see logically but that a person born in the United States in 1920 has just as much right to call himself an American as one born here in 1620. And further this right is just as logically passed on hereditarily now as it was then. In other words is there any reason not to accept little Gaetano Tomasino (let us say) as a friend and brother citizen, though his ancestors first landed on these fair shores in the early spring of 1920 instead of the late autumn of 1620, *provided,* and just as long as, *Gaetano behaves like an American?* If and when he does not then plainly something must be done about it. Practically, and in fact, that is the way in which Americans of all varieties, including that odd and rather dangerous creature now so much to the fore, the "100 per cent. American," have come into being.

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* I realize as fully as my Canadian friends the unfortunate element in the common usage of "American" with reference solely to the United States rather than to North America. Canadians are of course as much Americans as we are. But, in point of fact, because United States is not a term easily put into

Divorce.

Divorce is unquestionably a factor in the biology of human reproduction, and should therefore be considered in any general resumé of the subject. From the point of view of the present discussion, however, it is wholly impossible to do anything with this topic, since no data are available. In "Marriage and Divorce, 1916," a special report of the Bureau of the Census issued in 1919, the following statement is made:

> Two investigations with respect to marriage and divorce have been made previously by the Federal Government—one in 1887–88 by the Department of Labor, covering 20 years from 1867 to 1886, inclusive, and the other, in 1906–7 by the Bureau of the Census, covering the 20 years from 1887 to 1906, inclusive. These two investigations together covered a consecutive period of 40 years.

The results of the first of these studies are incorporated in the second. It thus results that the entire statistical knowledge of marriage and divorce for this country as a whole is contained in these volumes. And in no one of these are there any data about race or nationality. Consequently from our present viewpoint nothing can be done with the material.

IV.

NATALITY.

The most important data regarding births, from the viewpoint of the present discussion, have already been presented in the section

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adjective form, while America is, common usage has seized upon the last word of the official designation of this country rather than the first two, to use as the descriptive adjective for her citizens.

on marriage, as a result of dealing with *effective* marriages solely. It remains here to consider certain special topics not there dealt with. These topics are (*a*) fertility, (*b*) illegitimacy, (*c*) still-births.

A. *Fertility.*

The general fact that foreign-born women are much more fertile, under American conditions, than native-born is well known, and can be shown in many different ways. Perhaps the most striking way to set forth the broad fact is to show the relation of births to married women of child-bearing age in the population as a whole. This is done in Table VI, which is in rearranged form, a table given on p. 10 of Birth Statistics, 1919.

TABLE VI.

Showing the relation between the number of foreign-born women of child-bearing age and their contributions to the population.

State.	Per cent. of			
	Children of foreign-born mothers to total children of white mothers.			Foreign-born married females 15–44 to white married females 15 to 44.
	1919.	1918.	1917.	1910.
Rhode Island	†	53.59	55.76	49.94
Massachusetts	52.58	53.20	55.36	48.87
Connecticut	56.76	58.20	60.21	46.36
New York	48.99	49.10	51.64	42.71
Minnesota	21.90	22.25	24.86	33.99
New Hampshire	37.78	38.51	40.81	32.69
Pennsylvania	33.88	34.52	36.33	27.77
Michigan	29.11	30.08	32.46	26.45
Wisconsin	18.70	19.08	20.62	26.40
California	28.04	*	*	23.74
Washington	19.86	20.16	21.69	23.24
Maine	25.13	24.24	25.92	21.89
Utah	12.73	13.17	14.56	18.83
Vermont	20.71	21.38	22.47	18.11
Ohio	21.71	21.87	23.11	15.51
Oregon	12.98	*	*	14.81
Maryland	13.15	13.16	14.29	13.11
Kansas	6.54	6.12	6.91	8.12
Indiana	8.43	8.46	8.95	5.72
Virginia	2.06	2.06	2.05	2.35
Kentucky	0.82	0.77	0.86	1.55
South Carolina	0.58	*	*	0.90
North Carolina	0.30	0.23	0.34	0.47

* Not added to registration area until later date.

† Excluded because all transcripts of birth certificates for the year were not received.

From Table VI it is seen that in 12 of the 23 states in the table the foreign-born women were the mothers of a higher percentage of children than would be expected if they were no more fertile than the native-born women of the same age groups. In the case of Connecticut the percentage of births rises to 10 or more points higher than the percentage of representation in the population.

For one state, New York, Eastman (8) has made a very careful and thorough study of the birth-rates by race. Table VII shows some of his chief results.

TABLE VII.

Births to white mothers, according to nativity of mothers, in New York State, (exclusive of New York City) in 1916. (After Eastman.)

Nationality of mother.	Estimated population in 1916.	Number of births.	Number of births per 1,000 estimated population.	Crude birth rate of native country according to last report before war.	Date of last report before the war.
Total white	4,643,786	*102,834	22.1	....	....
Native white	3,777,685	64,889	17.2	....	....
Foreign-born white	866,101	37,914	43.8	....	....
English, Scotch, and Welsh	97,695	1,869	19.1	‡24.1 §25.5	1913
Irish	124,467	1,879	15.1	22.8	1913
German (includes German Poles)	171,435	2,421	14.1	27.5	1913
Italian	141,845	12,998	91.6	31.7	1913
Russian (includes Finland and Russian Poland)	82,195	7,281	88.6	44.0	1909
Austro-Hungarian (includes Austrian Poles)	81,256	7,307	89.9	‖31.3 ¶36.3	1912
Canadian	104,270	2,219	21.3	†24.0	1913
Other foreign-born	62,938	1,940	30.8	....	....

Commenting on this table Eastman says:

> The features of interest that at once attract the attention are the extraordinarily high rates of the Italians, Russians and Austro-Hungarians (which amount to from 100 per cent. to almost 200 per cent. higher here than in the mother countries), and the exceptionally low rates of the Irish and Germans. A study of the statistics of immigration as contained in the reports of the Com-

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[*] Nativity of mother unknown in the case of 31 births.

[†] Province of Ontario.

[‡] England and Wales.

[§] Scotland.

[‖] Austria.

[¶] Hungary.

missioner General of Immigration of the United States will reveal the probable cause of these remarkable differences. Of the total number of Italians, Austro-Hungarians and Russians, who were admitted into this country since July 1st 1880, 75.3 per cent. and 77 per cent. respectively, arrived here from July 1, 1900 to June 30, 1914, while of the total number of Germans admitted since 1880, 60 per cent. arrived prior to June 30, 1890 and only 19.4 per cent. since July 1, 1900. The exact data regarding the Irish were not obtainable from the records from which the above figures were extracted, but it is very probable that the percentages are more or less similar to those of the Germans, assuming that the difference of age upon arrival does not differ materially between the two nationalities. This means that the three nationalities in New York State first mentioned are mostly composed of young adults in the most productive period of life, while the Germans and Irish are mostly people past childbearing age, or at least in the later stages of that period when the birth rate is much lower. It is notable that the Canadians and British have a lower birth rate in New York than in their respective countries of birth. This may be due to the greater similarity of their age constitution to that of the native class and to their greater inclination and ability to adopt the American standard of living.

Attention is directed to the fact that 73.1 per cent. of all births to foreign-born women were to Italian, Russian and Austro-Hungarian mothers and that these races accounted for nearly 27 per cent. of all the births occurring in the State outside of New York City, although they furnished less than 7 per cent. of the total population.

From a strictly scientific standpoint these figures are not thoroughly reliable. For all practical purposes, however, there seems to be no adequate reason why they should not be used. If anything the birth rates of the foreign-born are probably too low, since it is likely that the percentage of each foreign nationality to the total population was not as great in 1916 as it was in 1910, owing to the practical cessation of all immigration during the years of 1915 and 1916. This would probably more than offset any increase that may have occurred in the foreign population of the State from an influx of these people from other states, attracted by better industrial conditions, etc. It may therefore be assumed, that whatever error there may be, would, if corrected, but emphasize the point that the birth-rate of native women in New York State is undoubtedly as low, or lower, than that of France, and that the birth rate of the foreign-born woman is almost twice as great as that of the native woman.

The above birth rates (17.2 for native women and 43.8 for foreign women) are not fairly comparable owing to the great differences between the age constitution of each element. There is not only a greater proportion of married women from 15 to 45 years of age among the foreign class (according to the 1910 U. S. Census it amounted to 70 per cent. as against 52 per cent. for native women), but a larger percentage of them are between the ages of 21 and 30, the period of greatest productivity.

To illustrate the point Eastman presents the table, here given as Table VIII.

TABLE VIII.

A comparison of the crude, legitimate and illegitimate birth rates of native and foreign-born mothers. (From Eastman.)

	Birth rates.		
	Births to native-born mothers.	Births to foreign-born mothers.	Excess percentage of births to foreign-born mothers.
Number of births per 1,000 inhabitants	17.2	43.7	154
Number of legitimate births per 1,000 married women 15 to 44 years of age	137.1	253.2	85
Number of illegitimate births per 1,000 single, widowed or divorced women 15 to 44 years old.................	2.1	3.2	52

To indicate the reliability of the New York figures Eastman compares them with Kuczynski's data for Massachusetts in 1895. The results are shown in Table IX.

TABLE IX.

A comparison of the birth rates in Massachusetts in 1895 and in New York in 1916. (From Eastman.)

	Massachusetts, 1895.	New York, 1916.
Births per 1,000 native population	17.03	17.2
Births per 1,000 foreign-born population	52.16	43.7
Births per native adult female population	* 48.78	† 48.6
Births per foreign-born females	* 107.29	† 104.2
Births per married native women of child-bearing age	* 142.47	† 137.1
Births per married foreign-born women	* 251.76	† 253.2

* Ages 14–50.
† Ages 15–44.

It is clear that the agreement is close. The same result has also been got by Hill (24).

Altogether there can be no doubt that in those parts of the country where a relatively large proportion of the population is foreign-born, the fertility of the foreign-born women is greatly in excess of that of the native-born. Almost, if not quite, the first biological result of Americanization is to reduce the fertility of the marriages. This is, of course, to be expected on all of the following grounds:

(*a*) The young immigrant woman is generally speaking a strong, vigorous, healthy animal—pioneering stock in fact. Even in these times it requires a person of more than the average gumption—both of body and of character—to pull up roots in the homeland and venture to a new world.

(*b*) The standard of living of the immigrant is relatively low, and this has virtually always and everywhere meant a high birth rate.

(*c*) The first broad economic result of Americanization is a raising of the standard of living. This makes itself felt in the first generation (native-born of foreign parents) in respect of the birth rate.

It would appear that this particular phase of the general problem of immigration, which causes some persons such great alarm, is one which in a generation or two adjusts itself quite automatically. Foreign-born women in this country do have a high birth rate, but their descendants very quickly slough off this attribute of barbarity, as it is regarded by some.

B. Illegitimacy.

While having no direct bearing upon our main problem, the question of illegitimate birth rates is an interesting one on its own account. It has been by many persons considered to be an index of morality, but most statisticians who have studied the question are inclined to question or deny entirely its significance in this regard. Eastman has shown (cf., Table VIII *supra*) that the illegitimate rate, *on the basis of single, widowed and divorced women*, is higher for the foreign than the native-born women in New York State. But it is also of interest to reckon the illegitimate rate on the basis of 1000 *total births*. The result of this method for the B. R. A. in 1919 is shown in Table X, which is modified from a table in Birth Statistics, 1919, pp. 16 and 17. Those states from which the returns are known to be incomplete are omitted, but their results are included in the figures for the Registration Area as a whole.

The illegitimate rate per 1000 total births is more than three times higher for native-born than for foreign-born mothers as a whole. For no one of the groups of foreign-born mothers does the illegitimate rate on this base approach in magnitude that for the native-born mothers. In the separate states the few exceptionally high rates for foreign-born mothers are based in every case upon fewer than 5 illegitimate births, in which case, of course, no reliance is to be placed upon the single rate by itself.

These facts disclose an interesting statistical paradox. Table VIII

TABLE X.

Number of illegitimate births (exclusive of stillbirths) per 1000 total births for white children, by country of birth of mother, 1919.

(Numbers are shown *in italics* where illegitimate births are less than 5.)

Area.	Country of birth of mother.													
	Total.	United States.	Foreign country.											
			Total.	Austria; includes Austrian Poland.	Hungary.	Canada.	Denmark, Norway and Sweden.	England, Scotland and Wales.	Ireland.	Germany; includes German Poland.	Italy.	Poland (not specified).	Russia; includes Russian Poland.	Other foreign countries.
Registration Area	12.0	14.9	4.1	3.9	5.1	10.0	5.5	8.6	8.7	5.6	1.5	4.1	2.1	3.7
Connecticut	9.1	15.0	4.4	6.3	*3.7*	8.9	11.2	*6.7*	4.7	—	2.5	7.5	3.1	*4.0*
District of Columbia	18.3	12.4	*2.6*	—	—	—	—	—	—	—	11.8	—	—	—
Indiana	11.6	12.3	2.3	3.7	*5.4*	—	—	*4.9*	—	*2.7*	—	*1.2*	—	—
Kansas	5.8	5.9	2.2	—	—	—	—	*9.3*	—	—	—	—	*2.3*	*3.8*
Kentucky	9.9	9.9	*2.3*	—	—	*47.6*	—	—	—	—	—	—	—	—
Maryland	18.6	20.9	3.0	*2.4*	—	—	*25.0*	—	—	*12.3*	—	*2.5*	*1.5*	*14.7*
Michigan	12.7	15.5	5.5	5.6	*2.4*	9.0	10.3	15.6	*11.0*	5.8	*1.6*	3.7	2.3	5.5
Minnesota	16.0	18.8	5.7	3.4	*3.7*	10.8	6.2	19.1	—	*3.3*	—	*7.5*	*4.5*	6.3
New York	9.3	14.7	3.8	3.8	6.2	20.1	4.9	12.0	9.1	5.6	1.2	5.6	1.8	6.0
North Carolina	14.8	14.1	—	—	—	—	—	—	—	—	—	—	—	—
Ohio	13.1	15.7	3.5	3.4	4.9	12.7	*5.5*	5.7	*4.0*	7.5	*1.1*	*1.1*	2.0	4.1
Oregon	13.6	14.5	5.8	—	—	*11.1*	—	*6.0*	—	*7.2*	*13.2*	—	*14.2*	*3.1*
Pennsylvania	16.3	22.1	4.6	4.1	6.4	*11.1*	10.4	10.0	12.7	9.1	1.9	5.6	3.3	5.3
South Carolina	16.9	16.9	—	—	—	—	—	—	—	—	—	—	—	—
Utah	7.8	8.4	3.7	—	—	—	*5.1*	*2.9*	—	*13.7*	—	—	—	*2.4*
Virginia	18.2	18.5	—	—	—	—	—	—	—	—	—	—	—	—
Washington	9.5	10.6	5.3	*6.7*	—	14.9	4.9	*4.3*	*7.6*	*3.8*	*3.1*	—	—	*1.5*
Wisconsin	11.8	13.4	3.9	3.2	*5.3*	*4.6*	*3.1*	*5.4*	—	6.1	*1.3*	5.3	*1.6*	*3.7*

shows that the illegitimate rate is higher per 1000 foreign-born women capable of having an illegitimate baby, than it is per 1000 native-born women in a similar social situation. But per 1000 total births the illegitimate rate is smaller for foreign- than for native-born mothers. The explanation is, of course, simple. There are so many more total births per 1000 foreign-born child-bearing women than per 1000 native-born that the illegitimate rate per 1000 births is bound to be low because the denominator of the rate fraction is large.

The essential point is that brought out in Table VIII. The foreign-born, unmarried, widowed or divorced woman is more likely to have a child than a similarly situated native-born woman. This probably does not mean better morals on the average in the latter case, but simply better judgment, which in turn means higher intelligence.

C. Stillbirths.

Data for stillbirths are not particularly accurate in the United States because of local differences in the legal definition of a stillbirth. Regarding this matter the Census Bureau (Birth Statistics, 1918, pp. 28 and 29) makes the following comments:

> Interpretation of the data relating to stillbirths must be made with extreme caution, as the term "stillbirth" is not used in the same sense in the various states and the percentage of the completeness of these reports is not known. The Model Law reads: A stillborn child shall be registered as a birth and also as a death, . . . provided that a certificate of birth and a certificate of death shall not be required for a child that has not advanced to the fifth month of uterogestation.
>
> Of the states in the birth registration area, Minnesota, New York, and North Carolina have similar provisions in their laws. The District of Columbia requires a certificate for a fetus "passed the fifth month." The Indiana law reads "seven months' gestation and over," and the remaining states use the term stillbirth, but do not define it.
>
> In Maryland special efforts have been made to secure uniform registration of stillbirths of even the earlier months of uterogestation, so that the data for this state are not comparable with those for other states. At first thought these differences in the various state laws seem to limit greatly the comparability of the data, but the fact that the figures of those states requiring reports only of children which have reached the fifth month approximate the figures of those states which do not define the term "stillbirth," warrants the conclusion that in the latter states only stillbirths of the later months are for the most part reported, so that the figures are fairly comparable. Moreover, it is undoubtedly true that stillbirths of the later months of uterogestation are well reported, because such stillbirths are interred, and burial cannot legally take place without a burial permit, issued upon receipt of the physician's certificate of death.

All data relating to stillbirths have been compiled from transcripts of stillbirths reported as births. Undoubtedly the total numbers of stillbirths would have been somewhat larger had data been compiled from transcripts of stillbirths reported as deaths, for foundling stillbirths would then have been included; but as most of the important facts relating to stillbirths are recorded on the birth certificates only, the Bureau of the Census gathered the information from this source.

For reasons, presumably connected with economy (most certainly of the false variety), all data on stillbirths are omitted in the 1919 Birth Statistics. We shall therefore have to take 1918 for the latest figures.

Table XI (from Birth Statistics, 1918, in rearranged form) gives the data as to stillbirths by country of birth of mother. To this table has been added a final column giving the average total fecundity of the several groups of mothers, as of the same year. The Registration Area is not quite the same for the figures of the last column and the next to the last, but the error so introduced is negligible for the use to which the figures are here put.

TABLE XI.

Births, stillbirths, stillbirths per 100 live births, and average number of children ever born, for white children by country of birth of mother, 1918.

Country of birth of mother.	Births.	Stillbirths.	Stillbirths per 100 live births.	Average number of children ever born.
United States	838,000	30,891	3.7	3.1
Total foreign	314,780	12,392	3.9	3.9
Canada	13,861	651	4.7	3.5
Ireland	14,038	620	4.4	3.3
England, Scotland and Wales	11,069	473	4.3	3.3
Italy	71,373	2,989	4.2	4.4
Russia (includes Russian Poland)	60,269	2,369	3.9	3.3
Austria (includes Austrian Poland)	62,111	2,384	3.8	3.9
Other foreign countries	22,355	835	3.7	3.6
Denmark, Norway and Sweden	11,624	413	3.6	3.6
Germany (includes German Poland)	15,414	559	3.6	4.6
Poland (not specified)	14,534	525	3.6	4.2
Hungary	18,132	574	3.2	3.8

The stillbirth rate is higher, on the basis of total live births, for foreign- than for American-born women in general. In the case of Scandinavian, German, Polish and Hungarian mothers the stillbirth

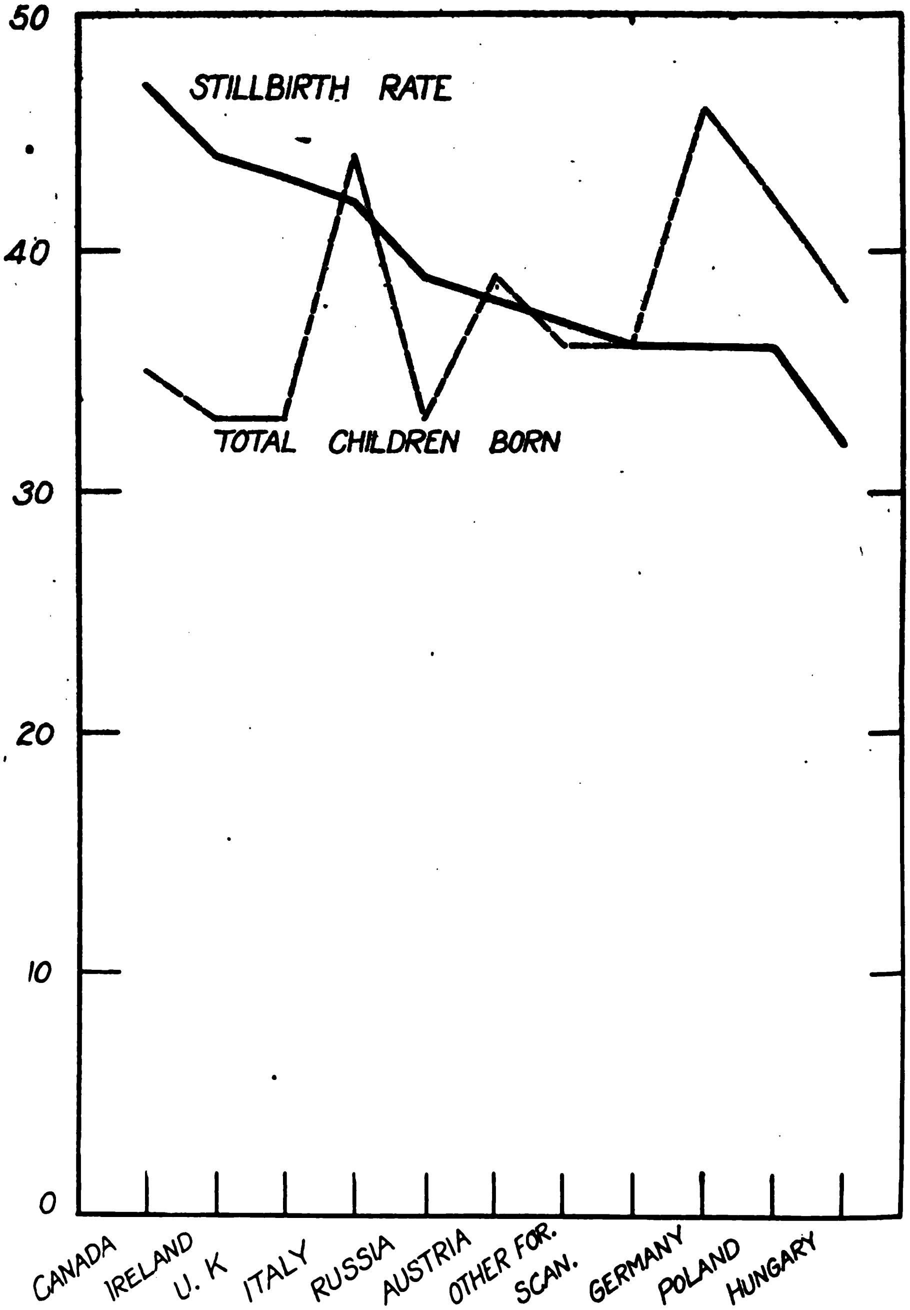

FIG. 11. Showing the relations between the stillbirth rate and the total number of children which have been born.

rate is lower than for the native-born mother. One's first thought is that the higher stillbirth rate of the other foreign-born mothers, as compared with the native-born, is due to the higher fertility of the former group. Comparing the fourth with the third column of Table XI gives little support, however, to such a view.

On the contrary, Fig. 11, which is plotted from the data of Table XI, seems rather definitely to indicate that the opposite relation holds on the whole. Namely, the more children a woman has had the less likely is the next birth to be a stillbirth.

V.

MORTALITY.

From published official data it is impossible to get any comprehensive or precise information as to the relative mortality of the different racial groups in this country. The fault is not so much with the original data contained in the death certificates, but with the fact that this material is not compiled, and by publication made available for the public information. The most that can be done is to learn something about the comparative mortality of the native- and foreign-born as total groups.

For the purpose of indicating what the facts in this restricted field show, Tables XII and XIII have been prepared. Table XII shows, for the six states, Connecticut, Massachusetts, Minnesota, New York, Pennsylvania and Wisconsin taken together, the population and deaths in 1910, divided according to whether the decedents were (*a*) native-born of native parents, (*b*) native-born but of foreign or mixed foreign and American parentage, and (*c*) foreign-born, and also by sex and age.

The six states chosen were taken because of their representative character in respect of different industries, and because of their large foreign-born populations. Together the states included in Table XII had in 1910 a total population of 25,205,393 individuals about each of whom age, sex, nativity, and parent nativity were definitely known. This is a respectable total number, and tends to give confidence in the results.

From the data of Table XII have been calculated death rates specific for age and sex for each of the three groups. These death rates are exhibited in Table XIII. Unless some arithmetical error has been committed in these calculations and overlooked, they represent

TABLE XII.

Population and deaths by sex, age and nativity, in six selected states, 1910.

Age.	Native-born males of native parentage.		Native-born females of native parentage.		Native-born males of foreign or mixed parentage.		Native-born females of foreign or mixed parentage.		Foreign-born males.		Foreign-born females.	
	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.	Population.	Deaths.
Under 1 year	133,312	17,414	129,877	13,284	151,852	24,298	147,861	18,403	1,678	202	1,546	190
Under 5	640,590	23,449	626,017	18,651	684,231	34,440	671,771	28,547	24,433	705	23,852	654
5–9	572,115	1,996	560,499	1,802	563,975	2,229	558,680	2,103	77,482	303	76,590	288
10–14	536,371	1,210	528,686	1,165	526,755	1,220	521,939	1,130	97,286	241	96,169	232
15–19	509,528	1,637	516,632	1,559	486,348	1,798	496,577	1,553	188,413	901	189,223	636
20–24	464,304	2,062	487,573	1,977	378,932	2,169	408,925	1,966	421,031	2,470	352,597	1,508
25–29	412,284	1,985	420,361	2,000	300,913	2,182	323,635	2,011	496,717	2,963	370,220	1,985
30–34	353,853	1,815	355,736	1,687	263,865	2,642	284,009	2,032	438,641	3,049	326,409	2,086
35–39	324,381	1,981	319,080	1,619	252,168	2,945	267,062	2,127	400,235	3,647	312,995	2,439
40–44	270,568	1,872	266,494	1,653	201,219	2,713	212,287	2,020	359,062	4,170	279,084	2,566
45–49	233,522	1,951	230,752	1,689	166,141	2,669	173,051	1,966	303,204	4,618	242,098	2,872
50–54	217,332	2,558	214,152	2,213	135,964	2,652	137,891	2,001	234,597	4,651	192,357	3,180
55–59	172,234	3,018	170,721	2,512	75,189	2,027	76,818	1,593	163,464	4,687	147,355	3,577
60–64	141,568	3,664	149,246	3,007	39,264	1,411	42,071	1,209	139,908	5,631	136,559	4,918
65–74	186,961	8,438	207,898	8,240	33,910	1,913	38,058	1,952	175,063	11,388	178,827	11,079
75–84	68,992	6,785	85,992	7,749	7,346	905	9,144	1,001	61,395	7,944	67,783	8,478
85–94	10,448	1,955	15,356	2,765	1,045	271	1,431	397	8,883	2,298	10,845	2,586
95 and over	285	92	591	181	35	13	48	13	349	156	498	208
Totals	5,115,336	66,468	5,155,786	60,469	4,117,300	64,199	4,223,347	53,648	3,590,163	59,822	3,003,461	49,292

a very close approximation to the true forces of mortality, operating in each defined group. The reader should not confuse them with crude death rates. They are true specific death rates, the specificity extending to the three variables age, sex and nativity with parent nativity.

TABLE XIII.

Specific death rates per 1,000 living of same sex and age, from Table XII.

Age.	Native white of native parentage.		Native of foreign or mixed parentage.		Foreign born white.	
	Male.	Female.	Male.	Female.	Male.	Female.
Under 1 year........	130.63	102.28	160.01	124.46	120.38	122.90
Under 5.............	36.61	29.79	50.33	42.53	28.85	27.42
5–9...........	3.49	3.21	3.95	3.77	3.91	3.76
10–14..........	2.26	2.20	2.32	2.17	2.48	2.41
15–19..........	3.21	3.02	3.70	3.13	4.78	3.36
20–24..........	4.44	4.05	5.72	4.81	5.87	4.28
25–29..........	4.81	4.76	7.25	6.21	5.97	5.36
30–34..........	5.13	4.74	10.01	7.15	6.95	6.39
35–39..........	6.11	5.07	11.68	7.96	9.11	7.79
40–44..........	6.92	6.20	13.48	9.51	11.61	9.19
45–49..........	8.35	7.32	16.07	11.36	15.23	11.86
50–54..........	11.77	10.33	19.51	14.51	19.83	16.53
55–59..........	17.52	14.71	26.96	20.74	28.67	24.27
60–64..........	25.88	20.15	35.94	28.74	40.25	36,01
65–74..........	45.13	39.63	56.41	51.29	65.05	61.95
75–84..........	98.35	90.11	123.20	109.47	129.39	125.08
85–94..........	187.12	180.06	259.33	277.43	258.70	238.45
95 and over.....	322.81	306.26	371.43	270.83	446.99	417.67

The data of Table XIII are shown graphically in Figs. 12 and 13.

The first point which strikes one from the examination of these specific death rates is that the infant mortality (death rate under 1) is *lower* for the foreign-born, in the case of both males and females, than for either of the other two classes. This result is a spurious one, arising from the following circumstances. To get entered in the deaths under 1, in this country, a foreign-born baby must have first been born abroad, survived the trip to this country and then died here. Now in view of the fact that the heaviest incidence of mortality in the first year of life is known to be always and everywhere in the first month of the first year, it necessarily follows that some unknown, but fairly large proportion of the new-born babies of families about to immigrate to the United States in 1910 died, either before the family left the old country, or while they were upon the ocean. Consequently they would never appear in our statistics. But there would be left to die in this country during the first year of life a smaller number of foreign-born babies, and furthermore a selected kind of babies, namely the survivors of the period of heaviest incidence of infant mortality. So we must conclude that the foreign-born

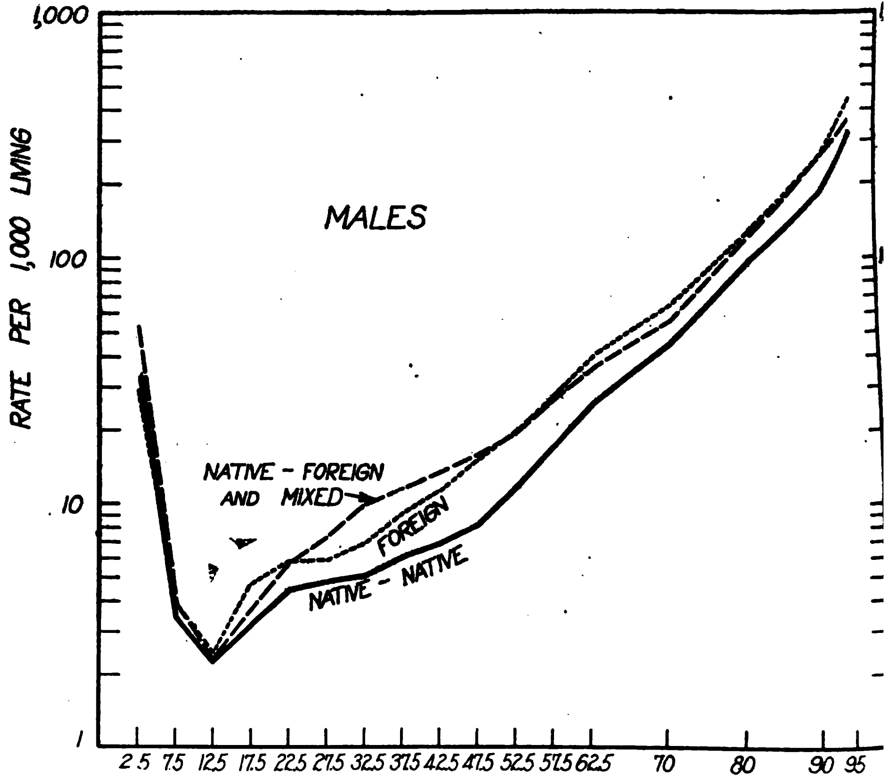

FIG. 12. Specific death rates for (*a*) native-born males of native parentage (solid line), (*b*) native-born males of foreign or mixed parentage (dash line), and (*c*) foreign-born males (dot line) in six selected states (see text) in 1910.

mortality under 1 is not accurately comparable with the native-born mortality in the same age group. The influence of this spurious element makes itself felt throughout the "Under 5" group.

After this period is passed the specific rates are *lower,* at all ages right away to the end of life, for the native-born of native parents than for either of the other two groups, in both males and females, with the single exception that the native-born of foreign or mixed parentage rate for females in the age group 95 and over is a little lower than the rate for native-born women of native parentage. No significance is to be attached to this slight deviation from the rule at the end of the curve, because all rates for that age group are subject to large probable errors because of the meager numbers involved.

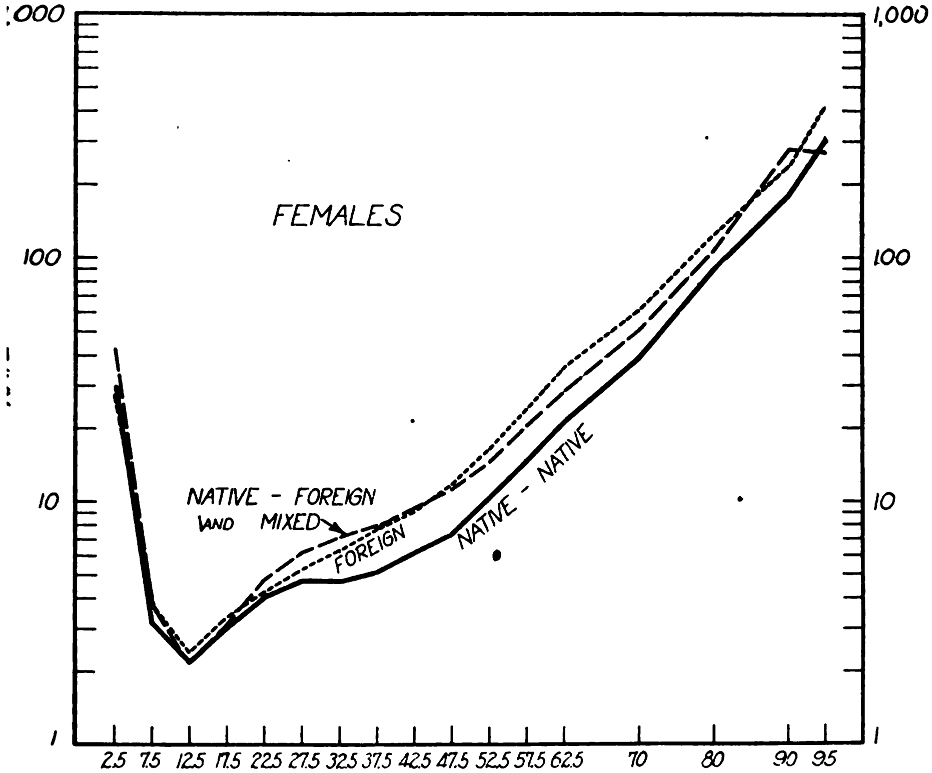

FIG. 13. Specific death rates for (*a*) native-born females of native parentage, (*b*) native-born females of foreign or mixed parentage, and (*c*) foreign-born females in six selected states in 1910. Significance of lines as in Fig. 11.

The superior estate of the native-born of native parents in respect of mortality undoubtedly arises from the combined action of the following factors: (*a*) *superior intelligence,* not perhaps in an absolute or abstract sense, but concretely in respect of awareness of and ability to grapple with the particular kind of environmental difficulties connected with living in the United States. This is probably the most important single factor. The native-born American of native parentage has a pretty fair fund of knowledge and tradition as to how best to contend with strictly American dangers—hygienic, economic and other.

(*b*) *Superior physical environment,* associated with a higher general standard of living. If one were obliged to put into a single statement the most general and important reason why genuine immigrants (meaning those intending to stay) now come to our shores, it would run, I think, in about this form: that above everything else the immigrant desires and intends to raise the standard of living of his family and descendants. After two generations have been born in this country the result has either been achieved or it has not. If it has not, the family that started the enterprise has, generally speaking, either died out or gone back home. There can be no question about the fact that, by and large, and in most parts of the country, the standard of living of the native-born of native parentage is definitely higher than that of either of the other two groups.

On the other hand, the relations of the specific mortality curves are, for the native-born of foreign or mixed parentage, most curious, and not entirely easy to explain satisfactorily. Taking the male data first, we see that in youth (from age 10 to about age 20) the specific rates are higher for the foreign-born. The curves cross at about age 22.5, and all through young adult and early middle life—up to age 52.5—the specific rates for the foreign-born are lower than those of the native-born of foreign or mixed parentage. From then on to the end of the life span the specific rates for the foreign-born are again higher than those for the native-born of foreign or mixed parentage.

The pertinent factors in the case appear to be as follows:

1. During childhood the foreign-born individuals probably receive on the whole less effectively good care in a hygienic sense than native-born children of parents of foreign or mixed parentage, on the ground that the recent immigrants understand our environment less well. Furthermore there is an extra occupational hazard upon foreign-born lives even in the childhood ages. So then the higher specific rates of childhood on the foreign-born are exactly what we should expect.

2. During early adult and middle life we have, apparently:

	Foreign-born.	Native-born of foreign or mixed parents.
a. Physical environment and standard of living	Worse	Better
b. Occupational hazard	Worse	Better
c. Innate biological constitution	Better	Worse
d. Racial constitution of the population	Better	Worse

Rubrics *a* and *b* need no explanation or argument, so far as I can see. Rubric *c* is meant to express the fact that immigration, even here and now, is a real form of pioneering. Only the relatively superior—in comparison with the group *out of which they come*—in physique, courage and general mental soundness have the initiative and strength of character to undertake the big adventure of starting anew in a strange country. That this selective action has its effect upon mortality, and that this effect is a considerable one, admits of no doubt (9).

Under rubric *d* is included whatever differences in race stock there may be between the foreign-born and the native-born of foreign or mixed parentage. Dublin and Baker (10) consider, on the basis of a study of New York and Pennsylvania alone, that the difference in the death rates that we are now discussing is practically entirely due to the "predominance of the Irish, German and British stocks among the first generations of Americans" in the age period 25–44. That this is a factor admits of no doubt. And it is an innate, biological one in last analysis. It does not seem to me, however, that Dublin and Baker have entirely demonstrated that it is the only, or even overwhelmingly important factor in the case.

In the study of the mortality of different race stocks in this country lies a wonderful opportunity for a piece of analytical work, the results of which could not fail, if it were carefully and sagaciously done, to throw a flood of light on some of our most pressing social as well as public health problems. What is wanted here is quantitative fact, not opinion. And it can be got, if only somebody sufficiently interested and public-spirited will finance the business.

So far we have been focussing attention upon the male curves. If we now turn to the female data, however, it is immediately apparent that whatever differences appear are essentially of degree not kind. In their general course the three female curves are essentially like the three male curves.

Altogether it may fairly be said that the exhibition of specific death rates, meager as is the specificity in respect of race, has served to define a large and important problem. If we could get compilations of deaths and populations separately for each racial group by age, sex, occupation, nativity and parent nativity, how much farther we could carry our knowledge of the factors influencing human mortality.

Already the attempt has been made to carry the analysis of racial factors in mortality in this country farther. Dublin and Baker (*loc. cit.*), by means of data specially obtained from the Census Bureau, have recently studied in a most interesting and profitable manner the mortality of different race stocks in New York and Pennsylvania. Dublin (11) earlier had made a similar study for certain race stocks in New York.

Dublin and Baker examined separately certain important causes of death. Their findings were as follows:

> *Tuberculosis of Lungs.*—The outstanding feature relative to pulmonary tuberculosis is the very unfavorable position of the Irish. Both sexes suffer extraordinarily from this disease during the greater part of life (25–84). At 25–44 the rates for Irish males are twice as high as for natives, and in the age period 45–64 the excess is even greater. The death rate among Irish males from tuberculosis continues high even at the advanced ages. The facts with regard to the Irish in the two states studied, are very different indeed from those in the native country, both as to the high rates obtained and the general configuration of the mortality curve. German and British males also show higher tuberculosis rates than the native-born at the older age. On the other hand, the Italians Russians and Austro-Hungarians present comparatively low tuberculosis death rates. At most of the age periods they are even lower than for the native-born. Italian females in New York show a striking exception to the usual rule in that their rates are higher than those for males, especially in early life. Otherwise there are no important differences in the several race groups with reference to this disease.
>
> *Cancer.*—Practically every foreign race group shows higher cancer rates than do the native-born. Italian males and females offer the only clear-cut exceptions. At the age period 45–64, foreign-born males show rates from 50 to 100 per cent. higher. The conditions among the Irish are strikingly bad. Among females at ages 45–64, the Germans and the British also show higher rates than do the native-born, but the excess is not as marked as it is among the Irish.
>
> *Organic Diseases of the Heart.*—Again the Irish present the highest rates, followed by the British and the Germans. The conditions prevailing among these from Austria-Hungary, Russia and Italy are, on the whole, very favorable with regard to this disease. It is very difficult to understand the reason for the aberrant death rates among the Irish in Pennsylvania and New York, for they are so much higher than are those for the native stock and for the Irish in their own country.
>
> *Pneumonia, all forms.*—From ages 45–84, the rates of the native-born are much the lowest. The Irish are again under the greatest handicap, their rates being about three times as high at ages 45–64. The rates for Italians are very unfavorable also at ages 45–84. The disadvantage of the Germans, British and Russians is less among females than among males. The position of the Austro-Hungarians is very much the same as the native-born at 25–64.
>
> *Bright's Disease.*—Again, we must record the very unfavorable position which the Irish occupy. From 25–84 years of age, the rates for this nationality are about 100 per cent. higher than for the native-born; among females, the excess is even greater. At ages 45–84, the rates from Bright's disease are high for Austro-Hungarians, Russians, Germans and British. The position of the Italians is quite favorable.

Violence (excluding suicide).—Deaths from this cause are largely from accident, and we find, of course, that the positions of the several race groups are determined by the extent to which they engage in occupations that expose them to such dangers. The figures are especially striking for Pennsylvania where large numbers of immigrants find employment in mills and mines. Males of all the important foreign groups show very high rates from this cause, the worst conditions prevailing among the Austro-Hungarians, followed by the Irish and Italians.

Their whole study Dublin and Baker summarize as follows:

1. Of the three main groups of the white population in Pennsylvania and in New York, (*a*) native-born of native parents, (*b*) native-born of foreign or mixed parentage, and (*c*) foreign-born, the first has the lowest mortality. This is true for both sexes and for virtually every age period, but it is most marked at the adult ages.

2. The foreign-born and the native-born of foreign or mixed parentage, agree much more closely with each other than with the native stock. An interesting exception presents itself, however, at ages 25–44, at which period the foreign-born have a great advantage over the native-born of foreign or mixed parentage. The reason for this is the predominance of the Irish, German and British stocks among the first generation Americans at this age period. After age 45, these two groups of the foreign stock are of the same racial extraction, and their death rates are in very close agreement.

3. The death rates of the component groups among the foreign-born vary considerably. The Austro-Hungarians, Russians, and Italians present altogether favorable conditions, while the British, Germans and Irish show death rates very greatly in excess. This is especially true of the Irish, whose mortality is about double that of the native stock. The rates for the Germans, British and Irish are much higher in America than in their own countries. Pulmonary tuberculosis, pneumonia and degenerative diseases including heart disease, Bright's disease, and cancer are largely responsible for this unfavorable mortality.

4. The findings of the previous study for New York State are confirmed. The unfavorable conditions of life and work among foreign races to which attention was directed in the study for New York are found to prevail in Pennsylvania as well. The facts emphasize the necessity for special public health work for the people of foreign origin. The much more favorable economic conditions under which they live in the United States than in their own countries should result in lower death rates. But in several instances, we find that this does not prevail; the facts indicate on the whole, deterioration rather than improvement. Is it possible that our immigrants are not representative of the best in their native countries? It has often been supposed that the immigrants comprised the most vigorous among their own people; the results, however, do not confirm this impression, but suggest many questions for further inquiry.

5. It is very important that a study similar to this one be carried out as soon as the results of the 1920 census are available, to determine whether any differences of importance have appeared in the interval of ten years.

Interesting and valuable as is the study of Dublin and Baker it is

TABLE XIV.

Infant mortality from principal causes and by the nativity of the mothers—1916. (From Eastman.)

	Total births.	Deaths under one year from all causes.	Inf. Mort.	Deaths from Principal Causes.									
				Communicable diseases (1–14 inc.).		Respiratory Diseases (28–29 and 86–98 inc.).		Gastro-intestinal diseases (103–104 inc.).		Premature birth, congenital debility and malformations (150–153 inc.).		All other causes.	
				D's.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort.	Deaths.	Inf. Mort.
Total mothers	103,530	9,912	95.7	471	4.5	1,530	14.8	2,265	21.9	4,435	42.8	1,211	11.7
Total white mothers	102,834	9,760	94.9	463	4.5	1,505	14.6	2,239	21.8	4,372	42.5	1,181	11.5
Total colored mothers	696	152	218.4	8	11.5	25	35.9	26	37.4	63	90.5	30	43.1
Native white mothers	64,889	5,648	87.0	229	3.5	684	10.5	1,096	16.9	2,943	45.3	696	10.7
Total foreign white mothers	37,914	4,112	108.4	234	6.1	821	21.6	1,143	30.1	1,429	37.7	485	12.8
Italian mothers	12,998	1,259	96.9	90	6.9	313	24.1	317	24.4	402	31.0	137	10.5
Russian mothers (excluding Russian Poland)	3,665	377	102.9	18	4.9	64	17.5	128	34.9	126	34.4	41	11.2
Austro-Hungarian mothers (excluding Austrian Poland)	6,345	822	129.6	41	6.4	165	26.0	273	43.0	268	42.3	75	11.8
Polish mothers (including German, Austrian and Russian Poland)	4,703	577	122.7	37	7.9	116	24.7	191	40.6	171	36.3	62	13.2
Total for group	27,711	3,035	109.5	186	6.7	658	23.7	909	32.8	967	35.0	315	11.4
British mothers	1,869	134	71.7	6	3.2	14	7.5	22	11.7	68	36.4	24	12.8
Irish mothers	1,879	182	96.9	8	4.2	19	10.1	40	21.3	82	43.7	33	17.6
German mothers	2,296	283	123.3	7	3.0	48	20.9	80	34.8	117	51.0	31	13.5
Canadian mothers	2,219	243	109.5	13	5.9	43	19.4	45	20.2	107	48.2	35	15.8
Other foreign-born mothers	1,940	235	121.1	14	7.2	39	20.1	47	24.2	88	45.4	47	24.2
Total for group	10,203	1,077	105.6	48	4.7	163	16.0	234	22.9	462	45.3	170	16.7

TABLE XV.

Principal causes of deaths of infants under 1 year of age showing percentages due to certain elements of the population. (From Eastman.)

	Total births.	Births per cent.	Total deaths under one year.		Causes of death.									
					Communicable diseases (1-14 inc.).		Respiratory diseases (28-29 and 86-98 inc.).		Gastro-intestinal diseases (103-104 inc.).		Prenatal causes, etc. (150-153 inc.).		All other causes.	
			Deaths.	Per cent.	D's.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.	Deaths.	Per cent.
Total white mothers	102,834	100.0	9,760	100.0	463	100.0	1,505	100.0	2,239	100.0	4,372	100.0	1,181	100.0
Native white mothers	64,889	63.1	5,648	57.9	229	49.5	684	45.4	1,096	49.0	2,943	67.3	696	58.9
Total foreign-born mothers	37,914	36.9	4,112	42.1	234	50.5	821	54.6	1,143	51.0	1,429	32.7	485	41.1
Italian, Russian, Austrian and Polish mothers	27,711	27.0	3,035	31.1	186	40.2	658	43.7	909	40.6	967	22.1	315	26.7
Other foreign-born mothers	10,203	9.9	1,077	11.0	48	10.3	163	10.8	234	10.4	462	10.6	170	14.4

plain that a great deal further remains to be done along similar lines.

A very interesting study has been made by Eastman (12) of the relation of parental nativity to the infant mortality of New York State. It brings out in such a clear manner the significance of what are perhaps the two most important elements in determining rates of mortality, namely, innate biological constitution, on one hand, and general intelligence, on the other hand, that it will be of value to examine the results in some detail. Tables XIV and XV (which are Tables V and VI of Eastman's paper) show the data.

Commenting on Table XIV Eastman says:

> The striking dissimilarity existing in the relative importance of the several main groups of causes, as distinguished between the native- and foreign-born mothers, is at once apparent even on a casual review. The infant mortality from communicable diseases was almost 75 per cent. greater among children of foreign mothers than among the babies of native mothers; from respiratory diseases it was over 100 per cent. greater, and from gastro-intestinal diseases the excess was about 78 per cent.; but the rate from prenatal and other causes peculiar to early childhood was higher among the native element by over 20 per cent. Pursuing the analysis still further, by dividing the total number of foreign-born mothers into the same two groups adopted in the preceding tables, we find these differences much accentuated when the native stock is compared with the group comprising the Italians, Russians, etc. The other foreign-born mothers, considered as a class, also exceeded the native mothers in the death rates from communicable, respiratory, and gastro-intestinal diseases, but the rates from prenatal, etc., causes were identical.
>
> Although the table gives us a very fair idea as to the relative prevalence of the several causes among certain classes of the population, it fails to show the percentage of the complete number of deaths from any particular cause that was attributable to each of the three classes of the population.

This relation referred to is brought out in Table XV. Regarding this Eastman says:

> Considering first the deaths of children of native white mothers, it will be noted that although they formed 57.9 per cent. of all the deaths from all causes, they nevertheless contributed only 49.5 per cent. of the total deaths from communicable diseases, 45.4 per cent. of all the deaths from respiratory diseases, and 49 per cent. of the total mortality from gastro-intestinal diseases. At the same time, 67.3 per cent. of all the deaths from premature birth, congenital debility, congenital malformations, accidents at birth, etc., were of children of these mothers. In contrast with these it is interesting to observe that, notwithstanding the fact that only 42.1 per cent. of all the white infant deaths occurred to children of foreign-born mothers, 50.5 per cent., 54.6 per cent. and 51 per cent. respectively, of the complete infant mortality from communicable, respiratory and gastro-intestinal diseases were referable to them. As to the mortality from

> prenatal causes, however, it is to be remarked that only 32.7 per cent. of the total from these causes occurred among this element in the population. This table shows also that the high death rates from the first three groups of diseases, incident to the children of the foreign-born, were particularly attributable to the Italians, Russians, Poles and Austro-Hungarians.
>
> We may therefore consider that our previous surmise has been corroborated, namely, that the chief causes of infant mortality among the native population originate for the most part in adverse prenatal conditions, but that among the foreign element, and especially the Italians, Poles, etc., the most frequent causes of death are communicable, respiratory and gastro-intestinal diseases.

The explanation of these results is undoubtedly in general terms as follows: The fewer deaths from prematurity and congenital defects among the children of Italian and Slavic mothers indicate that superiority of innate biological constitution which is generally associated with emigrating stocks. On the other hand, the high death rate among these infants from causes which Eastman classes as communicable, respiratory and gastro-intestinal is chiefly due to the mothers' ignorance. As Eastman says:

> It is not to be wondered at that they are the dominant causes among the foreign-born population, the majority of whom are poor, illiterate, without knowledge of English and almost wholly ignorant of the elements of modern sanitation, and inhabit, as a rule, the most congested districts of the large manufacturing centers.

Guilfoy (13) has also made an interesting study of the influence of race upon mortality in New York City. His results are in good accord with those of the other investigators which have been cited.

VI.

VITAL INDEX.

The writer (6) has elsewhere suggested the term "vital index" to designate that measure of a population's condition which is given by the ratio of births to deaths within a given time. It may fairly be said that there is no other statistical constant which furnishes so adequate a picture as this of the net biological status of a population as a whole at any given moment. If the ratio 100 Births/Deaths is greater than 100 the population is in a growing and in so far healthy condition. If it is less than 100 the population is *biologically* unhealthy. Depopulation may not be actually occurring, if there is a sufficient amount of immigration to make up the deficiency in births. But fundamentally and innately the condition is not a sound one from a biological standpoint, though under certain circumstances it may b from a social standpoint. It is curious, in view of the obvious significance of this constant, the vital index of a population, that so littl attention is paid to it by demographers. After much study of i (mostly unpublished) I am convinced that no single figure gives s sensitive a measure of the vitality of a nation, or any subgroup o people as this does. There appears to have been no adequate genera discussion of it since that of Wernicke (14) in 1889, and even h does not use it in the most effective manner or form. Sundbärg (17) proposed its use as a "measure of civilization" of different peoples Rubin (18) criticized Sundbärg, but only in respect of technique proposing as a measure of civilization D^2/B in place of D/B, wher $D =$ deaths and $B =$ births. During the present year Pell (25) ha dealt with the idea implicit in the birth/death ratio, but in a mos inadequate manner.

For the purposes and viewpoint of our present discussion thi constant is peculiarly fitted. One of our main problems is to see wha is to be the probable future course of the several racial elements i our population. No better indicator of this could be had than th vital indices of the different groups. Unfortunately we find ou selves restricted, just as in other parts of the discussion, by a lack o the raw data needful to undertake a properly detailed analysis. I will be necessary, as in all of the previous discussion, to make the bes of a somewhat bad case.

In Table XVI are shown four vital indices for urban, rural an total births and deaths of each state in the B. R. A. for the year 1915 to 1918 inclusive.

The significance of the several indices is as follows:

$$\text{Vital Index } A = \frac{100 \cdot (\text{Births of whites of native parents})}{\text{Deaths of all native whites}}$$

In this index the births and deaths come from an identical grou of the population. The children born were of course native, and thei parents were also native-born. The deaths were of native-born, i.e. the same group as the parents of the births. All racial element (white) are included in births and deaths, but all are Americans in the sense of nativity.

$$\text{Vital Index } B = \frac{100\ (\text{Births of whites, both parents foreign})}{\text{Deaths of foreign-born whites}}$$

Here again both births and deaths come from an identical group.

The births are children of foreigners in this country. The deaths are of foreigners in this country.

$$\text{Vital Index } C = \frac{100 \text{ (Births of negroes)}}{\text{Deaths of negroes}}$$

This needs no discussion.

$$\text{Vital Index } D = \frac{100 \text{ (Births of whites)}}{\text{Deaths of whites}}$$

This is for comparison with C. Both C and D are true vital indices, in the sense that the parents of the births in the numerator are drawn from the same population group as the deaths in the denominator.

Unfortunately on the basis of present published official compilations of statistics these four are the only significant vital indices which can be drawn up. For any really deep understanding of what the biological effect is of racial fusion, and of a new environment, on the net vitality of populations we ought to have a whole series of racially specific vital indices. Here again there is no practical hope of getting these from purely official sources. Some one must come forward and finance a comprehensive and thorough investigation along these lines from outside.

The facts about Indices A, B, C, and D are set forth in Table XVI. In this table a figure in *italics* indicates that the absolute number of births and deaths on which the index is based is in each case less than 100. It will be noted that there are few such cases, and that they are practically all among the negroes of the northern states.

This table presents many novel points of great interest. We may first compare vital indices A and B, which indicate the relative biological vigor of the native-born and the foreign-born populations in this country. Taking totals first we note that for each grouping and each year Index B is much larger than Index A. Except for the rural population B is more than twice as large as A. Generally speaking the foreign population produces in this country approximately two babies for every death. The native population (as defined in Vital Index A) produces only a small fraction over one baby for each death. In other words the native population, even when so broadly defined as by Index A, is in about the same state as France before the war, and not in as vigorous a state as the French population is now.

An examination of the same point by states is of interest. In Connecticut, in all but the influenza year 1918, Index B was approximately four times as large as Index A, and in 1918 it was three times.

TABLE XVI.

Vital indices of various elements in the population of registration states, cities in registration states, and rural portions of the registration states in the Birth Registration Area. (1915–18 inclusive.)

State and group.		1915—Vital Index.				1916—Vital Index				1917—Vital Index.				1918—Vital Index.			
		A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Connecticut	Cities	82.9	355.8	94.8	195.4	81.8	340.6	85.1	189.3	90.4	331.0	82.0	196.1	72.9	219.2	75.1	143.7
	Rural	75.7	292.0	*60.4*	149.0	73.5	282.9	*81.3*	146.4	77.8	293.2	*82.7*	149.2	65.7	212.2	65.0	120.2
	Total	80.5	339.7	86.0	180.9	79.2	326.6	84.3	176.7	86.4	322.6	82.1	182.8	70.8	217.7	73.4	137.3
District of Columbia	Total	117.0	97.5	85.2	123.0	119.7	93.5	87.3	125.9	126.6	103.7	85.5	131.7	102.1	78.5	64.7	104.2
Indiana	Cities	*	*	*	*	*	*	*	*	144.0	174.5	71.5	158.2	124.1	142.5	66.3	134.9
	Rural	*	*	*	*	*	*	*	*	172.5	54.3	59.1	166.3	153.2	53.1	53.8	149.0
	Total	*	*	*	*	*	*	*	*	162.7	121.8	68.2	163.3	142.8	108.5	63.3	143.6
Kansas	Cities	*	*	*	*	*	*	*	*	149.8	103.6	70.6	150.0	116.2	72.3	65.6	114.7
	Rural	*	*	*	*	*	*	*	*	223.7	58.1	74.5	208.2	190.4	50.5	67.4	177.9
	Total	*	*	*	*	*	*	*	*	207.3	68.6	72.2	195.3	171.9	50.1	66.4	162.2
Kentucky	Cities	*	*	*	*	*	*	*	*	135.8	26.6	47.4	123.1	105.6	25.1	34.7	98.5
	Rural	*	*	*	*	*	*	*	*	241.4	35.9	91.0	236.4	203.1	38.6	74.5	199.6
	Total	*	*	*	*	*	*	*	*	221.9	29.7	76.0	202.9	183.1	29.3	60.3	177.0
Maine	Cities	75.6	188.6	*71.4*	131.5	73.8	163.8	*25.0*	122.4	79.4	173.8	*75.0*	128.4	70.7	124.9	*53.8*	106.2
	Rural	105.6	156.0	*87.5*	136.4	105.7	146.2	*18.7*	135.9	115.0	161.6	*8.3*	145.5	96.5	112.5	*21.1*	119.9
	Total	98.8	169.0	*80.0*	135.1	98.1	151.3	*21.9*	132.3	106.3	166.7	*35.0*	143.0	90.2	117.8	*34.4*	116.2
Maryland	Cities	*	*	*	*	138.3	166.4	82.9	136.8	137.7	152.1	80.8	152.7	96.2	107.7	63.7	105.9
	Rural	*	*	*	*	177.0	90.1	128.6	173.5	173.3	82.6	125.1	168.7	129.8	67.6	92.8	126.3
	Total	*	*	*	*	157.5	144.9	106.8	164.6	155.1	132.2	103.1	160.1	111.9	96.8	78.8	114.9
Massachusetts	Cities	86.7	276.2	113.2	186.4	87.0	251.2	101.1	176.3	92.1	246.5	111.3	179.7	70.1	171.5	97.0	129.6
	Rural	80.5	226.4	70.6	145.1	79.0	202.1	73.5	135.3	77.4	207.4	128.4	135.6	63.0	147.8	86.2	104.8
	Total	85.1	267.1	105.8	177.0	85.1	242.3	96.0	167.2	88.5	239.6	113.3	169.9	68.5	167.4	95.4	124.5
Michigan	Cities	104.5	234.5	86.3	205.4	138.0	227.2	66.7	195.7	143.0	226.4	79.1	198.5	132.3	192.6	86.8	179.2
	Rural	182.2	143.5	83.1	197.9	172.2	140.5	68.1	186.7	171.7	139.4	64.1	185.3	155.9	123.8	77.0	167.4
	Total	165.0	187.8	85.2	201.2	157.2	184.4	67.1	190.9	158.8	184.2	74.5	191.5	145.2	159.7	84.1	173.0
Minnesota	Cities	173.0	166.3	59.9	211.5	163.2	148.2	51.4	194.5	170.8	136.3	67.7	194.5	137.0	105.1	61.1	156.4
	Rural	282.0	118.1	*18.1*	264.2	277.3	104.6	*88.2*	252.8	289.9	96.7	*50.0*	254.9	208.0	79.3	*42.9*	195.1
	Total	240.7	134.9	51.8	244.9	232.1	120.2	55.3	230.5	242.1	111.2	66.1	231.5	181.3	88.7	59.2	180.7
New Hampshire	Cities	70.3	293.7	*150.0*	163.0	72.4	248.4	*200.0*	153.9	69.2	239.5	*114.3*	147.1	60.9	164.1	*100.0*	113.7
	Rural	90.9	172.0	*60.0*	124.7	90.0	150.3	*30.0*	121.3	87.4	133.3	*60.0*	113.3	69.7	101.0	*80.0*	89.3
	Total	82.8	240.9	*110.0*	140.9	83.1	206.7	*69.2*	135.4	79.8	195.2	*91.7*	128.4	65.9	139.3	*71.4*	100.7
New York	Cities	88.4	273.5	94.9	170.5	88.5	255.4	101.6	172.5	95.6	246.0	96.3	175.2	79.4	187.1	84.5	137.1
	Rural	109.6	140.7	85.3	128.1	107.4	138.5	79.7	125.6	105.1	128.8	69.9	121.4	88.5	106.6	54.8	101.2
	Total	95.8	253.6	93.6	166.5	94.2	238.2	98.5	160.8	98.5	228.7	92.6	161.8	82.1	176.3	80.8	128.4

State and group.		1915—Vital Index.				1916—Vital Index.				1917—Vital Index.				1918—Vital Index.			
		A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
North Carolina	Cities	*	*	*	*	*	*	*	*	148.0	*60.9*	78.1	147.5	*99.1*	32.9	62.2	98.7
	Rural	*	*	*	*	*	*	*	*	266.8	*160.3*	190.3	270.4	223.7	47.0	145.0	224.4
	Total	*	*	*	*	*	*	*	*	255.4	106.3	173.1	258.2	209.2	41.6	33.8	209.3
Ohio	Cities	*	*	*	*	*	*	*	*	136.1	210.5	64.6	167.1	117.2	160.2	66.1	39.3
	Rural	*	*	*	*	*	*	*	*	157.5	113.3	73.8	156.3	138.2	98.5	70.1	137.5
	Total	*	*	*	*	*	*	*	*	147.0	182.2	67.2	162.0	127.7	143.3	67.2	138.5
Pennsylvania	Cities	117.2	273.3	95.2	179.2	110.4	253.8	87.6	166.5	114.6	243.1	74.4	166.3	81.1	153.7	59.3	112.5
	Rural	152.5	385.7	87.4	207.6	141.8	353.0	80.7	191.0	146.7	332.5	76.0	192.9	104.0	174.2	56.4	128.3
	Total	135.7	314.9	93.3	193.1	126.7	290.4	86.0	178.4	130.9	276.3	74.7	179.0	92.6	162.2	58.6	120.0
Rhode Island	Cities	74.3	219.5	83.2	158.3	72.5	216.4	76.1	152.7	79.2	217.8	89.5	159.2	64.0	172.5	91.6	126.0
	Rural	70.5	319.4	*65.8*	157.4	79.7	331.9	*113.6*	175.2	84.0	358.7	*123.8*	182.9	63.8	207.5	*29.8*	127.7
	Total	73.5	232.2	80.6	158.2	73.9	231.2	78.9	156.5	80.1	234.8	91.9	163.1	64.0	117.8	83.7	126.3
Utah	Cities	*	*	*	*	*	*	*	*	244.3	91.2	*140.0*	227.3	186.0	79.4	*75.0*	181.0
	Rural	*	*	*	*	*	*	*	*	390.7	97.1	*33.3*	338.9	312.7	71.6	*66.7*	274.2
	Total	*	*	*	*	*	*	*	*	339.4	94.7	*81.8*	297.8	265.4	74.8	*72.4*	238.5
Vermont	Cities	109.3	158.4	*100.1*	147.9	109.4	153.0	*100.0*	147.5	114.7	147.8	*100.0*	147.3	92.7	72.1	—	104.7
	Rural	121.3	138.4	*133.3*	147.2	110.7	135.5	*75.0*	134.8	114.3	134.7	*125.0*	136.6	96.5	91.3	*100.0*	111.5
	Total	119.9	142.5	*120.0*	147.3	110.5	139.0	*77.7*	136.7	114.4	137.3	*116.7*	138.2	95.9	86.5	*66.7*	110.4
Virginia	Cities	*	*	*	*	*	*	*	*	163.1	163.6	91.6	170.7	115.6	101.5	71.6	117.4
	Rural	*	*	*	*	*	*	*	*	255.3	125.4	159.2	252.6	200.7	103.6	137.0	200.1
	Total	*	*	*	*	*	*	*	*	233.4	144.7	139.2	232.6	177.7	102.4	117.1	176.8
Washington	Cities	*	*	*	*	*	*	*	*	169.1	123.2	*63.0*	184.8	132.2	84.3	*57.6*	140.3
	Rural	*	*	*	*	*	*	*	*	201.5	116.2	*42.3*	203.8	168.4	91.7	*56.8*	168.0
	Total	*	*	*	*	*	*	*	*	286.6	119.9	58.5	194.8	150.1	87.5	57.4	153.6
Wisconsin	Cities	*	*	*	*	*	*	*	*	178.4	142.2	75.0	194.8	143.2	115.9	*68.2*	156.9
	Rural	*	*	*	*	*	*	*	*	266.0	57.6	37.1	209.1	217.9	57.5	*65.2*	186.9
	Total	*	*	*	*	*	*	*	*	231.5	89.8	60.4	203.6	187.9	81.6	67.2	174.8
Totals	Cities	100.5	267.5	93.1	181.7	100.5	247.8	89.2	172.5	117.0	228.3	79.6	173.0	93.2	166.9	66.8	132.0
	Rural	141.1	215.4	82.5	179.0	137.7	199.9	109.0	170.1	177.7	156.5	146.2	187.4	144.8	118.8	118.4	150.8
	Total	117.8	252.4	91.4	180.7	116.3	234.1	94.2	171.6	148.1	205.2	114.3	179.8	118.8	151.8	93.7	140.6

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* Not in the Birth Registration Area in designated year.

In this state the native population, even when so broadly defined as to involve necessarily only one full generation in this country, did not, in any of the four years reviewed, come within a long distance of reproducing itself. It produced only about 0.8 of a birth for each death. Biologically that situation can have but one end.

The District of Columbia is in many respects demographically unique. Its abnormality is well reflected in the vital indices. *A*, while absolutely small, is still larger than *B* in each of the four years. The foreign population of the District succeeded in reproducing itself in only one of the four years, 1917, and then only barely so. Because of the obvious peculiarities of the population of the District no particular significance attaches to the result either way.

In Indiana, Kansas and Kentucky, in the two years for which data are available, the native population exhibits greater vitality than the foreign. (Index *A* greater than *B*.) The only exception is in Indiana cities, where the usual relation of *B* greater than *A* prevails. The foreign population makes a particularly bad showing in the rural portions of these states. It is by no means wholly a question of smaller numbers involved. For example in 1917, 4066 foreign whites died in Indiana, and only about 2000 more (exactly 6305) died in Connecticut. But whereas there were but 4952 births of which both parents were foreign in Indiana, there were 20,341 such births in Connecticut. The Indiana foreign population, in respect of racial stamina, age distribution and otherwise, is a less fit and vigorous population than the Connecticut foreign population. What is of more interest is the comparison of Vital Index *A* for these three middle western states with *A* for Connecticut. We shall return later to a more particular discussion of this, merely noting now that Index *A* is much higher in the middle west native population.

Maine is like Connecticut in the general trend of its figures for Index *A* and Index *B*. So also are the other New England states, Massachusetts, New Hampshire, and Rhode Island, with differences in degree in the several states. Vermont is the only New England state having Index *A* generally larger than 100.

In Maryland the native white population is reproducing itself less effectively in the cities than the foreign white, but much more effectively in the rural districts, making for the whole state a net balance in respect of biological vitality in favor of the native.

Michigan shows the same relation as Maryland as between urban and rural, but the net balance of the total, owing to the distribution

TABLE XVII.

States generally having

A less than 100.			*A* greater than 100.			*B* less than 100.			*B* greater than 100.		
Urban.	Rural.	Total.	Urban.	Rural.	Total.	Urban.	Rural.	Total.	Urban.	Rural.	Total.
Conn.	Conn.	Conn.	D. C.	Ind.	D. C.	D. C.	Ind.	D. C.	Conn.	Conn.	Conn.
Maine	Mass.	Maine	Ind.	Kan.	Ind.	Ky.	Kan.	Kan.	Ind.	Maine	Ind.
Mass.	N. H.	Mass.	Kan.	Ky.	Kan.	N. C.	Ky.	Ky.	Kan.	Mass.	Maine
N. H.	R. I.	N. H.	Ky.	Maine	Ky.	Utah	Md.	Utah	Maine	Mich.	Md.
N. Y.		N. Y.	Md.	Md.	Md.		Utah	Wis.	Md.	Minn.	Mass.
R. I.		R. I.	Mich.	Mich.	Mich.		Wis.		Mass.	N. H.	Mich.
			Minn.	Minn.	Minn.				Mich.	N. Y.	Minn.
			N. C.	N. Y.	N. C.				Minn.	N. C.	N. H.
			Ohio	N. C.	Ohio				N. H.	Ohio	N. Y.
			Pa.	Ohio	Pa.				N. Y.	Pa.	N. C.
			Utah	Pa.	Utah				Ohio	R. I.	Ohio
			Vt.	Utah	Vt.				Pa.	Vt.	Pa.
			Va.	Vt.	Va.				R. I.	Va.	R. I.
			Wash.	Va.	Wash.				Vt.	Wash.	Vt.
			Wis.	Wash.	Wis.				Va.		Va.
				Wis.					Wash.		Wash.
									Wis.		

of the population between urban and rural, is ir

Minnesota, Utah, Washington, and Wiscons degrees the same phenomenon we have seen in Kentucky. The native population is reproduc tively than the foreign, both in urban and rur

New York, Ohio and Pennsylvania behave in England states, the foreign populations in var the native in net vitality.

North Carolina and Virginia, the only south Table XVI, have small foreign populations, espec native population is on the whole definitely vitality than the foreign.

Summarizing, we may say that in the New the exception of Vermont) and New York the not reproducing itself, and in Vermont only b rural population of New York State is just ab tween births and deaths. The foreign populatio where showing a higher degree of vitality except Utah and Wisconsin. Also in the rural portions land their vital index falls below 100. The gen the influenza year 1918 are summarized in Tab

The relation between urban and rural pop the vital index is interesting. So far as the wh cerned, we note, having regard for indices *A* and rules hold generally. The native population has cities than in rural districts: the foreign popula relation, the *higher* index being for the city popul and with all factors included, as they are in the that the foreign population, as it is actually cons in respect of age, etc., is a biologically fitter p than is the native; while the native population conditions.

A point might well be made here by a critic of He might say: "It is all very well to show th tion, as actually constituted in this country, show than the native, but this fact may only mean th tion is more favorably distributed in respect o both in regard to deaths and to reproduction. tion contains a great many more people than th early adult life, which is the period of a higher rate and a low death rate, all the results you have shown might well appear from this cause alone."

This is an entirely valid criticism so far as any evidence we have yet presented goes. The vital indices of Table XVI are crude indices. We need age-specific vital indices for native- and foreign-born populations.

Let us put the matter in this way: Suppose that a gigantic corral were constructed with two compartments. Suppose that further there were put into one of these compartments, on a given date, all the native-born women aged 20–24 inclusive say, while into the other compartment were put all the foreign-born women in the country of the same ages. Suppose them all to be told that they were to stay there for one year, but that men could have free access to the corrals for purposes of reproduction. Finally suppose that similar corrals were constructed, and the women impounded in them, for each age group from say 10–14 at one extreme to 55 and over at the other extreme.

In any one compartment of any one corral during the year (*a*) some of the women would have babies, and (*b*) some of the women would die. If we kept statistical record of these events we could, at the end of the year, calculate the age specific vital index for each group of women. It would not be the general population vital index because no male deaths were included (and cannot be because of lack of published data). But it would be an age-specific vital index for the females as reproductive units.

TABLE XVIII.

Age specific vital indices for native-born and foreign-born women in B. R. A. 1919.

Ages.	Births from mothers born in U. S.	Deaths of native-born females.	Vital indices for native women.	Births from foreign-born mothers.	Deaths of foreign-born females.	Vital indices for foreign women.
10–14 ...	391	5,002	7.82	15	268	5.60
15–19 ...	77,048	7,763	992.50	10,768	759	1418.71
20–24 ...	258,876	11,854	2183.87	74,247	2,120	3502.22
25–29 ...	250,548	13,189	1899.67	102,429	3,317	3088.00
30–34 ...	166,777	11,813	1411.81	83,326	3,583	2325.59
35–39 ...	101,638	10,603	958.58	56,414	3,723	1515.28
40–44 ...	33,832	9,511	355.71	18,878	3,566	529.39
45–49 ...	3,202	10,092	31.73	1,866	4,120	45.29
50–54 ...	68	10,926	.62	54	4,968	1.09
55 and over..	26	96,919	.03	13	47,478	.02
Totals	892,406	187,672	....	348,010	73,902	....

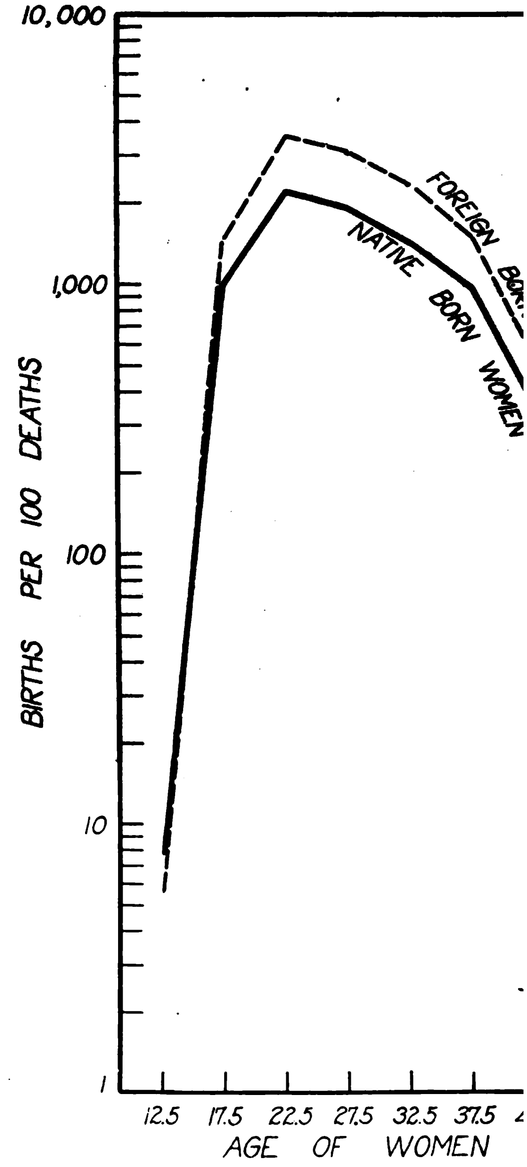

FIG. 14. Showing the differences in specific vita
foreign-born women in 1919. Solid line, native-born
born women.

The results of exactly such an experiment for the women of the B. R. A. in the year 1919 are shown in Table XVIII.

The figures in Table XVIII show plainly enough that at every age between 15 and 54 inclusive the foreign-born women have higher *specific* vital indices than native-born women. How much so is shown graphically in Fig. 14.

As a reproductive machine the foreign-born woman far excels the native-born. For each native-born woman dying between 20 and 24 years of age, the native-born women as a group produce approximately 22 babies. But for each foreign-born woman dying between 20 and 24, the foreign-born women as a whole produce 35 babies. It is in these five years that women, under conditions of life as now socially organized in the United States, do their best work biologically for the race, " best " being taken here in the sense of biological efficiency and economy.

So far as I am aware no attempt has been made hitherto to calculate age-specific vital indices. They picture, as exhibited in Table XVIII and Fig. 14, an extremely interesting biological fact. If we had such indices for populations of lower animals in different environmental situations, we should be in a position to know a great deal more than we now do as to the method of evolution. For it is the net balance between births and deaths which is the most significant information that can be had about the progress of the struggle for existence.

It may be objected in Table XVIII that we have put all births (both male and female) against only female deaths. The thought in doing this was that, after all, females have to produce *all* the babies whether the latter are boys or girls. If one wishes to postulate the problem in this way: how many new reproductive machines (females) do women of a specified age produce as a class for each similar reproductive machine lost by death, then of course one should take only female births in computing the specific vital indices. The result would be, of course, that the births and consequently the indices, Table XVIII, would be about one half as large absolutely as they are in that table, but the general *form* of the curve of Fig. 14 would be unchanged. What would be of great interest, but which we now unfortunately cannot do, is to make a table corresponding to Table XVIII, but for *males* instead of females. Why we cannot do it is because the birth statistics published do not separate fathers by both age and nativity. We can, however, get fathers of all nativities by age, and from that see

how the curve of age-specific vital indices run as compared with that for females.

The data for male age-specific vital indices Table XIX.

TABLE XIX.

Age specific vital indices for men in B. R. A. 1919.

Ages.	Births.	Deaths.
10–14	15	7,086
15–19	14,211	11,395
20–24	190,061	14,804
25–29	338,605	17,685
30–34	313,294	19,136
35–39	239,578	20,401
40–44	131,025	18,666
45–49	65,728	20,807
50–54	20,627	22,032
55 and over	10,123	163,447
Totals	1,323,267	315,459

The striking differences between the vital and those of Table XVIII are the higher values in the ages after 25 or 30, and the lower val specific vital index curve, as shown in Fig. 15 more spread out in general than the female curv be expected from the known facts about the productive capacities relative to age.

With the data for specific vital indices in to do would be to proceed to the calculation, B. R. A. as a whole, of vital indices *corrected* f of the several populations. Unfortunately, h in so many others, we are balked in the perfor enterprise because the raw materials are not publications as to make it possible. We shall direct methods to check up with more critical have drawn from the crude vital indices in Ta

The chief conclusions to which we have co amination of vital indices are these:

First, that generally index *B* (foreign pop index *A* (native population).

Second, that generally index *A* is higher fo populations.

Third, that generally index *B* is higher for urban than for rural populations.

We have now to ask this question: are these differences such in direction and magnitude as might reasonably be expected to arise merely from differences in the age constitutions of the several populations involved?

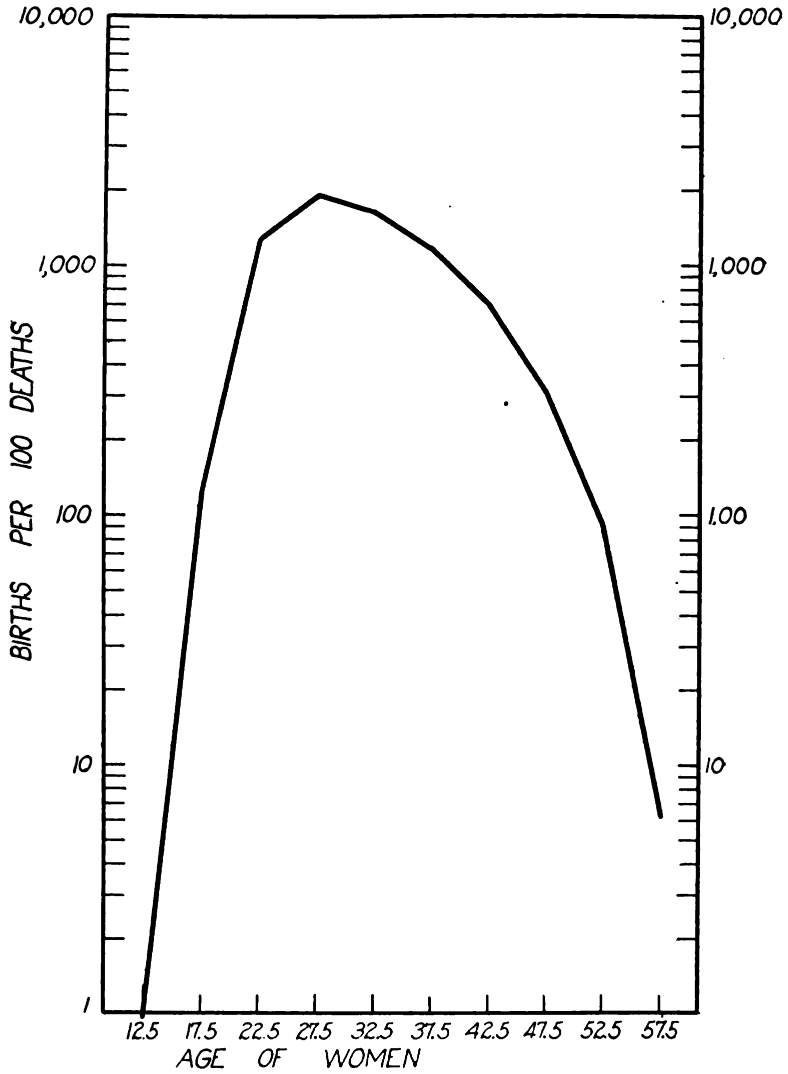

FIG. 15. Age-specific vital indices for males (see text).

As the first step in answering this question Table XX is presented. This shows for each state in the B. R. A., in 1917 and 1918, the percentage of women of ages 15 to 44 inclusive in the following divisions of the population in 1910: (*a*) urban native-born white, (*b*) rural native-born white, (*c*) urban foreign-born white, (*d*) rural foreign-born white. The ages 15 to 44 were chosen because Table XVIII shows that it is only within these years that the female age-specific vital indices rise to values greater than 100.

TABLE XX.

Percentage of women 15–44 years of age in the population in 1910.

State.	Order.	Native urban.	Order.	Native rural.	Order.	Foreign urban.	Order.	Foreig rural.
Connecticut	19	45.0	20	38.8	2	64.7	4	58.1
District of Columbia	4	52.7	......	......	15	54.5	......	......
Indiana	6	52.4	5	45.3	17	49.2	20	31.4
Kansas	7	52.3	1	46.3	20	46.6	16	40.6
Kentucky	3	53.8	6	44.4	21	34.2	19	32.0
Maine	20	44.9	16	40.9	8	61.3	6	56.0
Maryland	12	50.8	4	45.5	16	54.0	12	46.5
Massachusetts	18	45.3	19	40.1	3	64.4	3	58.8
Michigan	11	51.1	11	43.7	13	55.4	13	44.1
Minnesota	2	54.3	8	44.3	11	58.1	14	42.4
New Hampshire	21	42.9	18	40.3	4	63.4	5	57.2
New York	15	48.0	10	43.7	1	64.8	10	48.9
North Carolina	10	51.4	12	43.3	9	58.7	9	52.1
Ohio	8	52.2	7	44.4	14	55.0	15	41.9
Pennsylvania	14	48.6	14	42.1	7	62.3	2	63.8
Rhode Island	17	45.4	17	40.5	6	62.5	1	66.1
Utah	13	50.4	13	43.1	19	46.9	17	39.5
Vermont	16	46.3	15	41.6	12	56.0	11	47.1
Virginia	5	52.5	9	43.8	10	58.1	8	54.3
Washington	1	54.9	2	45.7	5	62.8	7	54.5
Wisconsin	9	52.0	3	45.6	18	48.6	18	36.6

From this table it is seen that, with some few exceptions, the following relations generally hold: The most favorable population in respect of the age distribution relative to vital index of females is the foreign urban, next comes the native urban, next the foreign rural, and last the native rural.

Turning now to our first conclusion we have the following question: if the proportion of females 15–44 were the same in the native-born population that it is in the foreign-born population would the former population, in any of its divisions, show as high values for the vital index as the latter population actually exhibits? The answer at once is that it could not, because Table XVIII shows that in each age class between 15 and 45 foreign-born women have a higher specific vital index than do native-born women, and if these indices were multiplied by the same population numbers the latter group would be bound to show a lower average vital index as a group. In other words, the foreign-born population has *both* a more favorable age distribution for the vital index, and also higher specific indices than the native-born.

The next question is: is the higher value of Index *A* for the rural native than the urban native population due to a more favorable age distribution of the former? As an aid to answering this question Fig. 16 is presented.

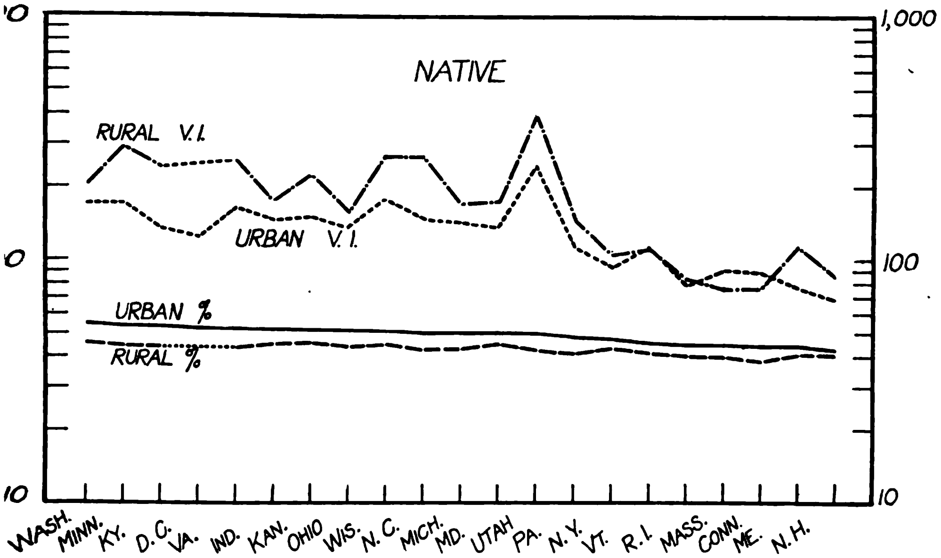

FIG. 16. Showing (*a*) percentage of native-born women 15–44 in urban population, (*b*) percentage of native-born women 15–44 in rural population, (*c*) Vital Index *A* for urban population, (*d*) Vital Index *A* for rural population.

The figure shows, first, the percentage of native women in the age period 15–44 inclusive in the urban population, the states being arranged in descending order of magnitude of this percentage. Next is plotted the percentage of this class of women in the rural population. It will be seen that in general this line runs parallel to, and everywhere *below*, the first. In other words, the age distribution of the rural population is, in every state included, distinctly less favorable to a high value of the vital index than is that of the urban native population.

Against these percentages taken from Table XX are plotted urban and rural values of Vital Index *A* for the same states, taken from Table XVI. The striking thing at once apparent is that, with the exception of the three states Vermont, Massachusetts, and Connecticut, the urban line lies everywhere *below* the rural, by an amount generally substantially in excess of that which separates the percentage lines. In other words, we see that while the native urban percentage of females in the child-bearing ages is higher than the native rural percentage, and would therefore lead to the expectation of higher vital indices in the former population, actually the vital indices show the opposite relation. In view of these facts there can be no doubt of the justice of our conclusion that rural conditions are substantially more favorable to the biological vitality of the native population than are urban conditions. Or, put in another way, the native population appears to be better adapted biologically to country than to city life.

The data are plotted on a logarithmic scale in order that slopes of the lines may be comparable. It is seen that in general the four lines run, in respect of their slopes, about parallel from the left margin of the diagram to and including Maryland. Utah's vital indices are much higher than would be expected from the age distribution of the child-bearing population alone. After that point clear to the right edge of the diagram, the relation between age distribution of the population and the values of the vital indices is obviously of a different order from that exhibited in the first half of the diagram. The New England states, for these are the ones here included, together with Pennsylvania and New York, have much lower vital indices for their native populations than the proportion of child-bearing women would entitle them to, on the basis of the showing of the other states. This can only mean that in the New England states especially, and to a less degree in New York and Pennsylvania, the age-specific vital indices of the native-born women must be extremely low. The native populations of these states are in a biologically unhealthy condition.

The next question is: is the general superiority of the foreign population in respect of the vital index in urban, as compared with rural environments, chiefly due to a more favorable age distribution of the child-bearing population in the former case?

Figure 17 puts the available data in graphic form.

The data plotted in this figure are the same, *mutatis mutandis,* as those in Fig. 16. The results, however, are quite different. Here the urban vital index line lies generally above the rural. In other words.

it is apparent that a substantial part of the superiority of the foreign vital index under urban conditions must be in fact merely due to the more favorable age constitution of the urban as compared with the rural foreign population. Probably in most of the states nearly all of the superiority is due to this factor. It is, however, of interest to note that the vital index lines in Fig. 17 do not parallel throughout their course the age percentage lines, in respect of their slopes. The last 8 or so states at the right end of the diagram show generally lower vital indices, both urban and rural, than would be expected from the age constitution of the population, on the basis of the other states included in the diagram. These are middle western states for the most part, where the foreign-born population, while socially more desirable perhaps than the foreign-born population of the more highly industrialized eastern states, is nevertheless a biologically less vigorous population.

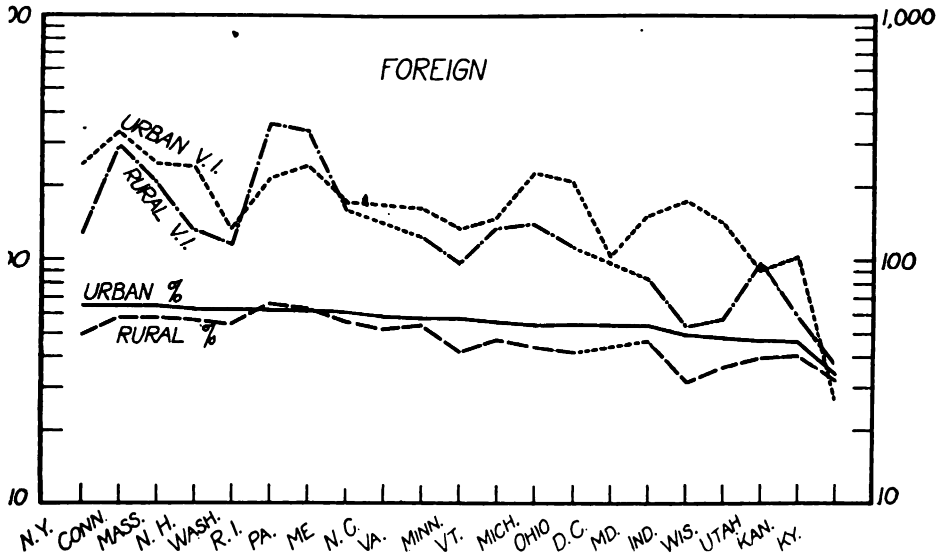

FIG. 17. Showing (*a*) percentage of foreign-born women 15–44 in urban population, (*b*) percentage of foreign-born women 15–44 in rural population, (*c*) Vital Index *B* for urban population, (*d*) Vital Index *B* for rural population.

The question of the secular trend of the vital index of the several components of our population is of great interest, but cannot be satisfactorily dealt with for two reasons: first, because only four years are available and one of these is highly abnormal because of the influenza epidemic; and second because the B. R. A. changed its composition radically by addition in the middle of the period. In consequence of this latter point the totals for 1917 and 1918 are not comparable with those for 1915 and 1916. When data have accumulated for a larger number of comparable years it will be profitable to go into the problem in detail. The writer hopes then to return to the matter.

There is one point of interest in the general connection discussed here, namely, the effect of the influenza epidemic in the vital indices. Comparing the totals of 1918 with those of 1917 it appears that:

Vital Index *A* (native white population) for cities fell 23.8 points or 20 per cent. of its value in 1917.

Vital Index *A* (native white population) for rural areas fell 32.9 points or 19 per cent. of its value in 1917.

Vital Index *A* (native white population) for total areas fell 29.3 points or 20 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for cities fell 61.4 points or 27 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for rural areas fell 37.7 points or 24 per cent. of its value in 1917.

Vital Index *B* (foreign white population) for total areas fell 53.4 points or 26 per cent. of its value in 1917.

From these figures it is evident that the epidemic reduced the net vitality of the population from 1/5 to 1/4 of what it was in the preceding year. This was a sharp blow. Further it appears that rural populations were slightly, but probably not significantly, less damaged relatively, in a biological sense, than urban populations. The foreign population was definitely harder hit biologically than the native. This might reasonably have been expected from all that we know of the general hygienic ignorance of this element of the population.

Let us now turn to the examination of Vital Index *C* for the negro population in comparison especially with *D* (total white population) and *A* (native white). The material includes only three southern (or border) states with large negro populations, namely North Carolina, Virginia and Maryland. In a number of the northern states, however, the negro population is large enough to furnish births and deaths in sufficient magnitude to give reliable indices.

The immediate and striking thing which our indices exhibit in comparing *C* and *D* is the low value of the former compared with the latter. Indeed one may say generally that, except in the rural districts of the southern states, practically never does the vital index of the negro population rise to a value of as much as 100. But plainly enough any population with a vital index under 100 is a dying population. Such a population is bound, in the fullness of time, to disappear completely, if nothing whatever is done about the case. Nowhere in *cities,* even in the southern cities, does the value of the negro vital index get to 100 in a fairly normal year such as 1917.

Even in rural portions of the B. R. A. the negro index does not approach in magnitude the total white index nor the native white index for the same communities. This is true in southern states as well as in northern. It would be difficult to find a more complete and critical demonstration than that furnished by these indices of the fact that the negro is biologically a less fit animal, *in the American environment physical, social and general,* than the white. It is idle to argue that if the negro were given a better chance, in a broad environmental sense, he might exhibit a degree of biological fitness, as measured by the vital index, equal or superior to that of the whites. Nobody knows whether that is true or not. And the important things are the facts as they are. Under conditions *as they are,* Nature, by the slow but dreadfully sure processes of biological evolution, is apparently solving the negro problem in the United States, in a manner which, when finished, will be like all of Nature's solutions, final, complete and absolutely definite. Just in direct proportion as the negro becomes anything but an agricultural laborer in the southern states does he hasten the time of his final extinction in this country. If he elects, which he will not, to become *exclusively* a farm laborer in the southern states, either for himself or somebody else, he must strictly limit his birth rate, which again he will not do, to the point where the numbers of his population will not exceed the economic demand and necessity for that particular kind of population, namely negro farmers. It would appear that, by nature, the negro is biologically fitted only to be in this country just precisely what he was before the Civil War, namely, an agricultural laborer in the South. This does not mean, of course, that there is any biological justification for the institution of slavery. On the contrary, as the South learned to its bitter cost, slavery is a most unstable and unbiological institution. But subtract slavery from the issue, as it happily has been subtracted, and the certainty of the biological conclusion cannot be shaken, so far as the experience of the last 50 years may be taken as indicative, and that is a fairly long time.

It is of interest to examine the comparative effect of the influenza epidemic on negro and white populations. Taking totals as before for 1918 as compared with 1917 we have:

Vital Index *C* (negro population) for cities fell 12.8 points or 16 per cent. of its value in 1917.

Vital Index *C* (negro population) for rural areas fell 27.8 points or 19 per cent. of its value in 1917.

Vital Index *C* (negro population) for total areas fell 20.6 points or 18 per cent. of its value in 1917.

Vital Index *D* (total white population) for cities fell 41.0 points or 24 per cent. of its value in 1917.

Vital Index *D* (total white population) for rural areas fell 36.6 points or 20 per cent. of its value in 1917.

Vital Index *D* (total white population) for total areas fell 39.2 points or 22 per cent. of its value in 1917.

The figures show that biologically the negro population of the B. R. A. was distinctly less severely damaged by the epidemic than the white population. The relation between urban and rural areas is exactly the reverse for negroes of what it is for whites. The rural negro population index was reduced more by the epidemic than the urban negro index, both absolutely and relatively.

VII.

CONCLUSION.

Out of the mass of statistical detail which has been presented in the preceding sections what sort of a general picture emerges? Have we answered, on the basis of vital statistics, our initial question as to what *kind* of a population this country is likely to harbor in the future, near or remote?

It must be regretfully stated that we have not been able to answer this question in any final or particularly satisfactory manner. I think, however, that it may fairly be said that we have shown that the responsibility for this failure is not primarily ours. It arises, as has been specifically pointed out in many places, because in the first instance the existing official information is not tabulated in such a way as to make possible many of the inquiries we should like to undertake and which are absolutely essential, if we are ever to have any deep understanding of our demography. In the second place, some information which is needed is not collected at all.

So far as the first of these difficulties is concerned, I am of the opinion that but one solution is practically feasible or possible. It is for some private foundation or group of individuals, who may be sufficiently interested, to come forward and finance an investigative organization for a period of years, to work in cooperation with the Census Bureau (a cooperation which the Census Bureau would probably gladly enter into). What this organization would do would be, in the first place, to tabulate in various new ways the raw data as to births and deaths, and in the second place proceed, after the preliminary tabulations were finished, to an exhaustive biometrical analysis of the data. The project is, from the standpoint of expense, by no means an impossible one, and I can think of no investigation likely to have such far-reaching anthropologic, eugenic, social, and even perhaps economic consequences, as this would. It is as true today as it was a quarter of a century ago when the eminent statistician, Dr. G. B. Longstaff (15), said in a meeting of the Royal Statistical Society, devoted to a discussion of the official statistics of the United States, that:

> As a theater of statistical investigation at the present time, no country in the world was so interesting as the United States, but with the imperfect tools at command they were unable to deal with a number of problems, quite unequalled elsewhere for magnitude and interest

This is not the place to present detailed plans for such an organization as that suggested, whether for its budget or its specific problems. The most I can hope for is that the discussion set forth in these pages may excite sufficient interest in the whole matter to make it profitable to outline specific plans for a real investigation.

Incomplete as our picture is as compared with what we should like to have, it still is a fact, I think, that the material which has been presented in these pages does give a little clearer and more comprehensive view than we have hitherto had of present tendencies, working in that great struggling mass of more than one hundred millions of individuals, who collectively make up the soul and body of these United States. Let us endeavor to summarize briefly what our findings are.

In the first place our population has indubitably passed the point of inflection of its growth curve, and from now on we may confidently expect an ever-increasing pressure of population on the means of subsistence, to use Malthus's classical phrase. The vital question is what kind of people are to inherit the blessings which Providence has so lavishly bestowed upon our portion of the North American continent? All cannot have them. There is not room enough. Man still breeds so fast that at all too frequent intervals he resorts to force to determine whether his or some other particular kind of people are going to enjoy the pleasures of continued existence.

The struggle may be varied in its manner. Economic pressure may in the long run be as effective an eliminant as machine guns. In general, unless forcibly prevented—which means finally by murder and sudden death—that people will inherit the earth and the fullness thereof which has habitually the highest vital index. The advocate of birth control as a solution of the problem of population should remember this, and draw from it the logical conclusion that if, for any reason whatever, he does not want the people who have the highest vital index to be the inheritors, he must be prepared to do something a good deal more violent than merely to control the birth rate of his own kind of people which is, in practical effect, about all that he has done so far. And he must not forget that people who have a high vital index are apt as a group to be pretty good fighters, in a technical military sense.

In the United States at the present moment certain broad facts seem to be outstanding in their significance for the future. They are:

1. That newly arrived foreigners rather speedily fuse effectively with the stocks already here, to a degree much greater than is presupposed, at least in most popular discussions of the subject of immigration and related matters. The temperature of the melting pot is higher than we generally give it credit for being. It really melts to a definite and significant degree.

2. That immigrant stocks are in the stark, raw business of reproduction vastly superior to native stocks, even to native stocks which have been here only a generation or two. Beyond this point there is no definite evidence, but all that can be inferred from the meager data available seems to indicate that it is a general rule that the farther we get in the fusion process from pure fresh immigrant stock the lower the vital index becomes. The end of the series is represented by the native stock of New England which is simply passing away, with considerable rapidity.

3. That native stock does better, in a biological sense, under rural than under urban conditions, while the opposite is true for immigrant stock.

From the standpoint of *quality* of the population in respect of health, intelligence, and all round efficiency, it is suggested that the wisest policy from now on for the country to pursue would be one which might be called intermittent or periodic immigration. Let all immigration of any sort whatever be rigidly and completely prohibited for a period of say 20 years, to be followed by a period of say 10 years of free immigration, with only such restrictions physical, economic and criminal, as we have in the immediate past imposed. During the period of no immigration the foreigners of the last influx and their children would become sensibly assimilated and fused into the American population. For the most part, they would have become Americans in a real sense of the word. Such a plan would further provide for that periodic inflow of new blood, of low living standards, which seems to be both an economic and social necessity, at least to civilizations which are still in the stage of industrial development and exploitation. Finally such a plan would, so far as one can foresee, postpone the period of pressing over-population as long as it can humanly be postponed, without the necessity of resorting to internal interferences of dubious practicability.

The United States has been, from its beginning, a gigantic experiment in human genetics. The experiment has gone on now three centuries, roughly speaking. When I compare the net result, as indicated by the human product now here—the product counted in the 1920 census—with the much more nearly pure-bred peoples of Europe, I cannot force myself to take that pessimistic outlook that I am told by some of my " 100 per cent. Nordic " friends that I ought to. It seems to me, looking at the matter as a biologist, that a real, distinct, unique *American* people has evolved in the course of the experiment and is still continuing to evolve. Further, as people go, it is not a bad people. Its most interesting and valuable feature is that it is still changing and evolving. I can find no manner of bitter feeling or even regret that the pure English-Scotch-Welsh stock of the original settlers will eventually not be the dominant element in the complex of American germ-plasms that it has been in the past, though personally I am wholly of that stock, and am, for three centuries, an American. Such a state of affairs was, in the natural course of events, bound to come about. There cannot, by any possibility whatever, be anything approaching biologically pure race stocks in this country a century hence. There are practically none now, with the exception of the Jews, and there is every present reason to believe that even they will be far less pure in 2021 than they are in 1921. How far we have progressed in only about one generation, in point of time, in racial fusion, is indicated by the comparison of a careful estimate of the racial stocks in the country in 1872 with conditions now existing. In the Journal of the Royal Statistical Society in 1872 (16), it was estimated that the following figures gave the percentages of the different race stocks in the population at that time:

	Per cent. in population
Original British Stock	46
Irish	16
German	13
African (negro)	12
Recent British	8
French, Spanish and others	5
	100

The kind of people who will survive and run the affairs of the country, say a couple of centuries hence, when population pressure will be intense, will, I think, not be Englishmen, or Slavs, or Jews, or Italians, but *Americans*, of that type which has shown the greatest adaptability to the problems which life in this part of North America has presented. I think they will be just as gentle, as high-minded, as clever, as honorable, and as independent as any people on the face of the earth.

VIII.

SUMMARY.

1. It is shown that the population of the United States (*a*) is growing according to a curve of the form

$$y=\frac{be^{ax}}{1+ce^{ax}},$$

(*b*) has passed the point of inflection of this curve, and (*c*) is rapidly approaching its asymptote.

2. The problem of the future is the *quality* of the asymptotic population. To estimate this from past conditions is one of the purposes of this paper.

3. It is shown that there are three general criteria by which the biological attributes of a population may be estimated or measured. These are: (1) somatic physical, (2) somatic psychological, and (3) biostatistical. These are defined and discussed. This paper deals

solely with the third of these criteria, including marriages, divorces, births, deaths, and the birth/death ratio.

4. Racial assortative mating in marriages effective in producing offspring in 1919 is discussed.

5. For the entire B. R. A. the percentage of amalgamation or fusion of foreign-born stocks with native-born, as compared with racially like effective matings of all sorts, as indicated by effective marriages in 1919, is just under 11.5 per cent.

6. The amount of racial amalgamation or fusion going on in the several states of the Registration Area is proportional in the most direct and close way to the amount of foreign-born white stock in the local population.

7. The same result holds if the percentage of fusion matings of foreign with native stock is taken upon the base of pure native matings, instead of all pure matings as before.

8. In 10 out of 23 states there are *more* effective mixed matings of the amalgamating type (i.e., foreign × native) than there are of racially true foreign × foreign matings.

9. In the three states Massachusetts, New York, and Connecticut, there were in 1919 as many or more matings of the type foreign-born × foreign-born as there were of the type native-born × native-born, *effective in producing offspring.* In these three states as large or larger additions to the future population were made in 1919 by the foreign-born as by the native-born. Further, in three other states, New Hampshire, Pennsylvania and Maine, the effective foreign × foreign matings were more than half as numerous as the effective native × native.

10. The foreigner in this country is much more apt to marry an American-born person, if he does not marry one of his own race, than he is to marry some other foreigner not of his own race.

11. In 1919, there were in the B. R. A. 78 racially different types of mating effective in producing offspring, not separating sexes of either mated partners or offspring. 65 per cent. of the births were from native × native matings, 10 per cent. of the children had one parent native-born and the other foreign-born, leaving approximately 25 per cent. of the births having both parents foreign-born.

12. In those parts of the country where a relatively large proportion of the population is foreign-born, the fertility of the foreign-born women is greatly in excess of that of the native-born. Almost, if not

quite, the first biological result of Americaniz
fertility of marriages.

13. The illegitimate rate is higher per 100
capable of having an illegitimate baby than
born women in the same social situation. But
the illegitimate rate is smaller for foreign- than
ers. The explanation of this apparent parado:

14. The stillbirth rate is higher, on the bas
for foreign- than for American-born women in g
Scandinavian, German, Polish and Hungarian
rate is lower than for native-born mothers. .

15. After infancy and early childhood the
death rates are lower at all ages for native-born
for either native-born of foreign parents, or
tailed discussion of mortality and race stock is

16. The biological vigor as indicated by
births/deaths) of the native-born population is n
the foreign-born population. In the New Engla
mont) and New York, the native population is
and in Vermont only barely so.

17. The native population has a lower vital
rural districts; the foreign population shows tl
higher index being for the city population.

18. A study of age-specific vital indices for
a reproductive machine the foreign-born womai
born. For each native-born woman dying bet
of age, the native-born women as a group proc
babies. The corresponding figure for foreign-bc

19. The peak value of the vital index for v
group 20 to 24, inclusive. For men the peak
25 to 29.

20. Age and sex corrected vital indices are

21. Rural populations were slightly, but
cantly, less damaged, relatively in a biologi
populations by the influenza pandemic. The f
definitely harder hit biologically by the pand

22. Except in the rural districts of the
tically never does the vital index of a negro
value of as much as 100. Nowhere in cities, e
does the value of the negro vital index get to

23. Biologically the negro population of the B. R. A. was distinctly less severely damaged by the influenza epidemic than the white population.

24. Some eugenic bearings of the study are discussed.

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A NOTE ON THE INHERITANCE OF DURATION OF LIFE IN MAN.*

BY RAYMOND PEARL.

(Received for publication January 2d, 1922.)

Experimental studies on *Drosophila* have definitely demonstrated that in that form duration of life is inherited in accordance with Mendelian principles (1, 2). In the case of man all the evidence so far available regarding the inheritance of duration of life has come from statistical studies, notably those of Pearson (3) and his associates, and Alexander Graham Bell (4, 5). This sort of evidence, while entirely cogent as to the main fact of the inheritance of this character, is not such as to make possible the ready determination of the mode of the inheritance, whether Mendelian or other.

Since the publication of my Lowell Lectures on the biology of death, I have received from a number of correspondents bits of data upon one or another phase of the problems therein discussed. Amongst these contributions was an extremely interesting one from a southern lady regarding the facts about duration of life in her own family. This note is based upon the information so received. For obvious reasons names cannot be given here, but I may say, as bearing upon the trustworthiness of the data here set forth, that my informant is a trained observer along biological lines, and is well known for her contributions in one field of biology.

The pedigree shown in Fig. 1 may be first presented, and then the probable meaning of the facts it shows, discussed. It should be noted that in this pedigree ♂ denotes as usual a male individual, ♀ a female, and ○ an individual known to have existed, but about whom I have no further information, even as to sex. The numbers standing above and to the right of each sex sign are the designatory numbers (in lieu of names) of persons included in the pedigree. The numbers

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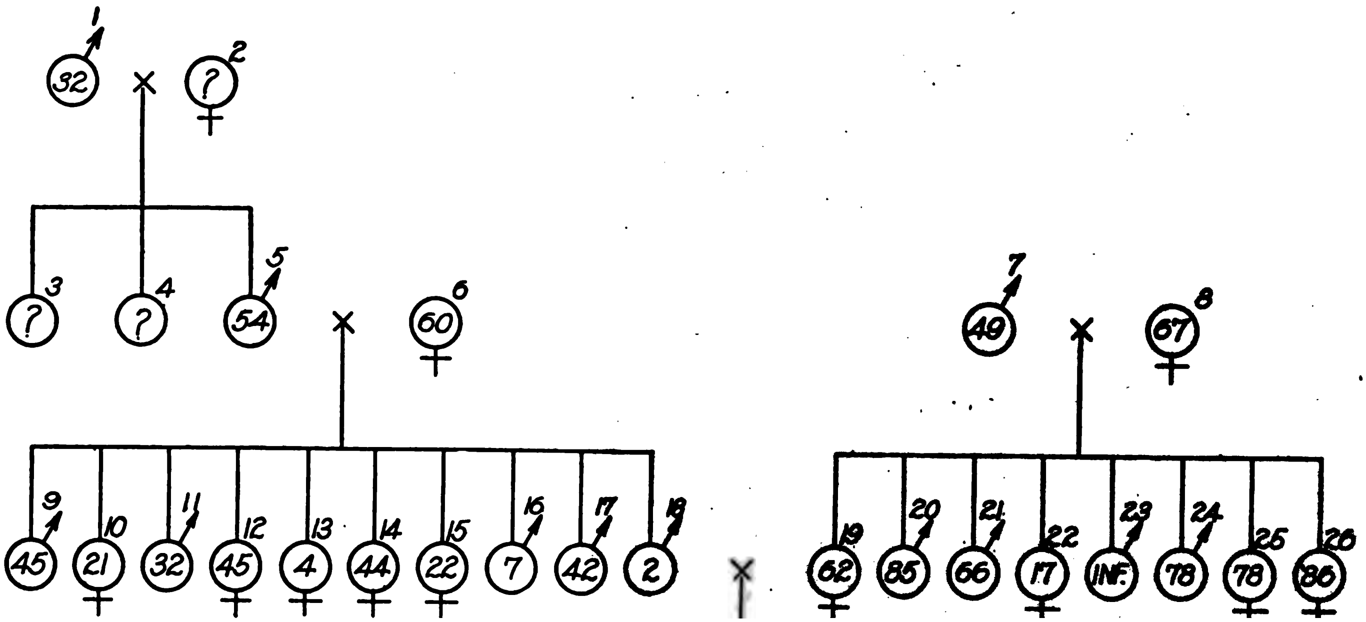
* Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 47.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within the circle of the sex sign give the ages at death of the individuals in years. All persons shown in the pedigree are dead with the exception of two marked "liv." below the numbers denoting their ages. One of these is my informant, to whom I am greatly indebted, both for furnishing me with the facts and for permitting their publication.

About individuals 3 and 4 no information is at hand. Their father died at the age of about 32 when they were small children. I furthermore have no information about the ancestry of individuals 2, 6, 7 and 8, except that individual 19 was reputed to "belong to a family with a remarkable record for longevity." The ages at death of both individuals 7 and 8 undoubtedly are under their potential longevity. No. 7's death "may be regarded as the nature of an accident, since he was a victim of yellow fever during the epidemic that visited Savannah in 1820." "His wife" (no. 8) "died at 67 of typhus fever contracted by remaining on the plantation to aid in caring for the sick negroes during a violent epidemic of the disease in 1852. As I remember her, she was a woman of wonderful energy and activity up to that time."

TABLE 1.

Survivorship of parents and offspring in pedigree Fig. 1.

Surviving up to	Number surviving to specified time are					
	A. Father's sibship (♂ P).		*B.* Mother's sibship (♀ P).		*C.* Offspring sibship (F_1).	
	Absolute.	Per cent.	Absolute.	Per cent.	Absolute.	Per cent.
Birth...............	10	100	8	100	8	100
10 Years............	8	80	7	87.5	7	87.5
20 "	8	80	6	75	7	87.5
30 "	6	60	6	75	7	87.5
40 "	5	50	6	75	7	87.5
50 "	1	10	6	75	7	87.5
60 "	1	10	6	75	6	75
70 "	1	10	4	50	3	37.5
80 "	0	0	2	25	2	25
80 and over.........	0	0	2	25	2	25
Mean duration of life*	33.7 years		59.1 years		60.9 years	

* Counting the "infant" as aged 1, and taking the two living at present age.

The data of the pedigree are arranged in the form of survivorship figures in Table 1. Column *A* gives the survivorship relations of the family containing individuals 9 to 18 inclusive; Column *B* gives the same material for the family containing individuals 19 to 26 inclusive; and Column *C* the family containing individuals 27 to 34 inclusive. On account of the small numbers involved no attempt is made to smooth in any way the l_x lines. They are merely the observed frequencies of survival.

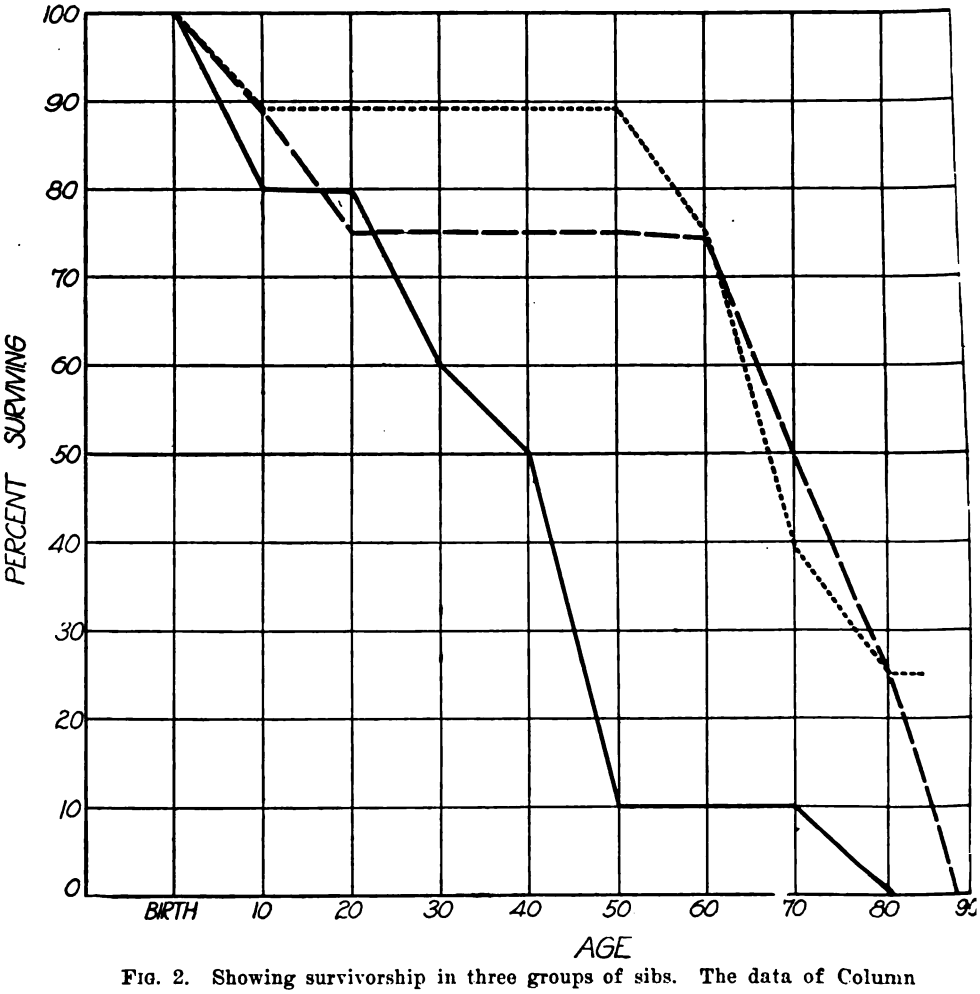

FIG. 2. Showing survivorship in three groups of sibs. The data of Column *A* of Table 1 are given as a solid line, those of Column *B* as a dash line, and those of Column *C* as a dot line.

The percentage columns of Table 1 are exhibited graphically in Fig. 2.

It is apparent from the table and diagram that the interest of this case lies in the fact that it represents the crossing of a short-lived with a fairly long-lived stock. In the father's sibship only 10 per cent. survived to age 50, in the mother's sibship 75 per cent. survived to that age, and in the offspring sibship 87.5 per cent.

Making due allowance for the irregularity of the survivorship lines of Fig. 2, consequent upon the small absolute numbers, the resemblance of this diagram to what one gets in a controlled Mendelian experiment on duration of life in *Drosophila* (cf. Fig. 4, p. 149, in my paper on the Biology of Death, VI, loc. cit.) is striking.

No conclusion can be drawn from a single pedigree of this sort, of course, but the case suggests the desirabality of analyzing extensive genealogical material in a similar manner. This I hope to be able to do later, upon material now accumulating in this laboratory.

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SEASONAL FLUCTUATIONS OF THE VITAL INDEX OF A POPULATION[1]

BY RAYMOND PEARL

DEPARTMENT OF BIOMETRY AND VITAL STATISTICS, JOHNS HOPKINS UNIVERSITY

Communicated, February 14, 1922

In earlier papers the writer[2] has discussed various aspects of the birth-death ratio, or vital index of a population, including age and sex specific vital indices, racial and secular fluctuations, etc. Table 1 of the preceding paper by Pearl and Burger[3] gives the necessary data for finding, for one population, that of England and Wales, the course of the seasonal fluctuation of this important demographic constant. It is the purpose of this note to set forth biometrically the facts on this point, from the basic data mentioned.

Table 1 gives the frequency distributions for variation in the value of the vital index of the population of England and Wales in each of the four quarters of the year, ending, respectively, on March 31, June 30, September 30, and December 31, during the years 1838 to 1920, inclusive. The source of the data is given in Pearl and Burger already cited.

The significant biometric constants from table 1 are presented in table 2. It is apparent that there are a few highly aberrant observations in

TABLE 1

FREQUENCY DISTRIBUTION FOR VARIATION IN THE VITAL INDEX IN THE FOUR QUARTERS OF THE YEAR

VITAL INDEX	QUARTERS ENDING			
	MARCH 31	JUNE 30	SEPT. 30	DEC. 31
65– 69	—	—	—	—
70– 74	—	—	—	—
75– 79	1	—	—	—
80– 84	—	—	—	—
85– 89	—	—	—	—
90– 94	—	—	—	—
95– 99	—	—	—	—
100–104	—	—	1	—
105–109	1	—	—	—
110–114	—	—	—	—
115–119	3	—	—	—
120–124	3	—	—	1
125–129	1	—	—	1
130–134	3	2	—	4
135–139	9	3	3	4
140–144	11	2	—	7
145–149	13	2	3	5
150–154	9	7	5	9
155–159	13	6	5	11
160–164	10	5	10	9
165–169	2	11	9	17
170–174	3	9	8	7
175–179	—	11	8	4
180–184	—	9	6	2
185–189	—	10	3	—
190–194	—	2	4	—
195–199	1	1	8	1
200–204	—	2	1	—
205–209	—	1	3	—
210–214	—	—	1	—
215–219	—	—	4	—
245–249	—	—	1	—
Total	83	83	83	83

TABLE 2

BIOMETRIC CONSTANTS DEDUCED FROM TABLE 1

QUARTER ENDING	MEAN	STANDARD DEVIATION
March 31	147.06 ± 1.21	16.32 ± .85
June 30	169.89 ± 1.21	16.35 ± .86
September 30	175.43 ± 1.66	22.39 ± 1.17
December 31	156.46 ± 1.27	17.14 ± .90

the series, but it has not been thought best to discard or adjust them in this first treatment of the subject. Consequently it is to be understood that all the values given in table 1 were used in computing the values in table 2.

From these data the following points are to be noted:

1. The vital index has the lowest mean value in the quarter ending on March 31, the winter quarter. In that period the birth incidence is relatively low and the death incidence relatively high.

2. Next in value to this, but standing 9.40 ± 1.75 points above it, is the mean vital index for the autumn quarter ending December 31. The difference being 5.4 times its probable error, may be regarded as significant.

3. The spring quarter, ending June 30, shows the next higher mean, being 13.43 ± 1.75 points above the winter quarter.

4. The highest value of the index falls in the summer quarter, when births are most frequent and deaths least so. The mean value, however, lies only 5.54 ± 2.05 above that for the spring quarter, a difference which cannot be regarded as significant.

5. In variability of the vital index, the first two and the last quarters of the year, all exhibit significantly the same status. The vital index is distinctively more variable in the summer quarter, the difference in standard deviations when this quarter is compared with that ending June 30, amounting to 6.04 ± 1.45. This may be regarded as significant, being 4.2 times its probable error.

It thus appears that the extremely close compensatory relation between birth rate and death rate, which Pearl and Burger[3] have shown to hold in annual figures, does not obtain within the single year. Instead there is a well-marked statistically significant intra-annual, or seasonal fluctuation of the birth-death ratio.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 51.

[2] Cf. particularly Pearl, R., "The Vitality of the Peoples of America," *Amer. J. Hyg.*, **1**, 1921 (592–674).

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THE VITAL INDEX OF THE POPULATION OF ENGLAND AND WALES, 1838–1920[1]

BY RAYMOND PEARL AND MAGDALEN H. BURGER

DEPARTMENT OF BIOMETRY AND VITAL STATISTICS, JOHNS HOPKINS UNIVERSITY

Communicated, February 14, 1922

The senior author[2] has suggested the term "vital index" as a convenient designation of the function

$$V = \frac{100 \ Births}{Deaths}$$

which measures more effectively than any other demographic function yet devised the essential biological fitness of a population, in the sense of organic evolution. For a population V is the direct measure of survival value. If $V > 100$ the population is not only surviving but growing, whereas if $V < 100$ the population is starting on the road to elimination, and must eventually disappear unless the vital index gets above 100. A detailed presentation of the facts regarding the vital index of certain elements of the American population has lately been published.[3]

In examining the effect of the Great War upon the growth of population one cannot fail to be impressed by the fact that the return of the birth/death ratio (vital index) to a normal or even super-normal value after the marked dislocation produced by the war, and at its end the superimposed influenza pandemic, was extraordinarily rapid. Indeed the senior author[4] was led to remark, after examining the matter in the chief belligerent countries, that "Altogether, these examples, which include the effects of the most destructive war known to modern man, and the most devastating epidemic

TABLE 1

RATIO *V*, OF 100 BIRTHS TO DEATHS IN ENGLAND AND WALES IN EACH QUARTER OF THE YEARS 1838 TO 1920, INCLUSIVE

	QUARTERS ENDED					QUARTERS ENDED					QUARTERS ENDED			
YEAR	MAR.	JUNE	SEPT.	DEC.	YEAR	MAR.	JUNE	SEPT.	DEC.	YEAR	MAR.	JUNE	SEPT	DEC.
1838	116	134	157	140	1866	142	150	153	158	1894	155	186	201	180
1839	138	146	157	141	1867	145	178	176	158	1895	141	179	174	161
1840	134	143	148	135	1868	166	184	147	160	1896	163	185	181	168
1841	135	151	164	150	1869	153	159	166	149	1897	164	179	169	170
1842	141	155	150	148	1870	143	168	155	151	1898	154	183	166	169
1843	144	150	167	150	1871	151	167	159	144	1899	157	182	151	152
1844	142	160	163	143	1872	155	173	170	176	1900	132	166	175	167
1845	137	153	177	163	1873	163	174	178	160	1901	158	182	169	167
1846	162	166	136	128	1874	157	176	169	150	1902	153	179	209	168
1847	122	130	136	123	1875	132	164	174	159	1903	170	195	207	169
1848	116	150	160	144	1976	162	179	180	177	1904	157	192	178	165
1849	145	150	100	139	1877	171	170	195	177	1905	160	192	195	172
1850	147	168	171	159	1878	158	177	172	155	1906	169	189	181	166
1851	149	160	165	150	1879	145	167	210	160	1907	143	187	216	161
1852	152	158	151	152	1780	152	186	167	165	1908	153	201	208	170
1853	137	147	160	140	1881	163	186	196	177	1909	143	189	218	172
1854	144	168	136	133	1882	159	182	186	163	1910	163	203	218	168
1855	123	155	177	153	1883	158	171	189	167	1911	156	188	158	169
1856	164	173	173	164	1884	170	181	167	165	1912	154	188	216	170
1857	157	170	160	146	1885	157	168	191	173	1913	148	187	199	172
1858	136	158	160	133	1886	147	184	179	168	1914	149	182	197	159
1859	144	166	161	155	1887	153	176	178	163	1915	125	154	180	134
1860	149	157	190	158	1888	149	179	199	169	1916	136	165	193	134
1861	143	172	170	159	1889	158	183	180	165	1917	109	136	172	136
1862	149	173	187	150	1890	136	171	180	139	1918	118	144	147	67
1863	145	160	154	155	1891	145	139	192	156	1919	75	137	197	195
1864	135	161	161	144	1892	120	176	197	168	1920	197	208	245	180
1865	138	167	160	148	1893	160	177	160	147					

TABLE 2

GROUPED DATA FOR VITAL INDEX AND CRUDE BIRTH RATE

PERIOD	BIRTH	DEATHS	100 BIRTHS / DEATHS	GENERAL BIRTH RATE PER 1000
1838–1839	956,361	681,744	140.28	31.0
1840–1844	2,600,288	1,756,431	148.04	32.2
1845–1849	2,797,329	2,003,657	139.61	32.6
1850–1854	3,080,095	2,030,528	151.69	33.9
1855–1859	3,300,929	2,126,461	155.23	34.3
1860–1864	3,560,830	2,263,769	157.30	34.9
1865–1869	3,830,527	2,438,121	157.11	35.3
1870–1874	4,100,856	2,541,625	161.35	35.5
1875–1879	4,399,070	2,623,391	167.69	35.6
1880–1884	4,451,771	2,591,038	171.81	33.8
1885–1889	4,450,173	2,620,108	169.85	32.0
1890–1894	4,486,912	2,778,642	161.48	30.5
1895–1899	4,611,116	2,771,151	166.40	29.6
1900–1904	4,691,038	2,739,365	171.25	28.5
1905–1909	4,637,271	2,613,992	177.40	26.7
1910–1914	4,411,823	2,519,713	175.09	24.2
1915–1919	3,623,894	2,685,456	134.95	20.8
1920	957,994	466,213	205.48	25.5

since the Middle Ages, furnish a substantial demonstration of the fact that population growth is a highly self-regulated biological phenomenon. Those persons who see in war and pestilence any absolute solution of the world problem of population, as postulated by Malthus, are optimists indeed. As a matter of fact, all history definitely tells us, and recent history fairly shouts in its emphasis, that such events make the merest ephemeral flicker in the steady onward march of population growth."

In view of the fact that this function V has so quickly returned to normal, or approximately normal, values after the recent disturbances it is a matter of considerable interest and significance to the student of problems of public health in the broad sense, of population growth, and of human ecology, to see how precisely the self-regulatory mechanism of populations operates over long periods of time. To this end we have examined all the existing continuous statistics of England and Wales, and desire to present the results in this paper.

Table I gives the vital index (100 births/deaths) for the population of England and Wales, in each quarters of the years 1838 to 1920 inclusive. The data from which the computations were made were taken from official sources.[5]

The immediately striking feature of this table is the extreme constancy of the values as one runs down the columns. In order to appreciate this fact fully, however, it is necessary to resort to graphical presentation. As the data of table I are too detailed to make such an examination of the trend feasible within the space of a single printed page, the data have been combined in quinquennial periods in table 2. In this table are given, for each 5 year period, the total births, total deaths, vital index, and crude birth rate. The latter figures have been calculated directly from the birth and population figures and differ slightly (but in no wise significantly) from the crude birth rates given in Annual Reports of the Registrar-General, chiefly because of the fact that we have used a different quinquennial grouping.

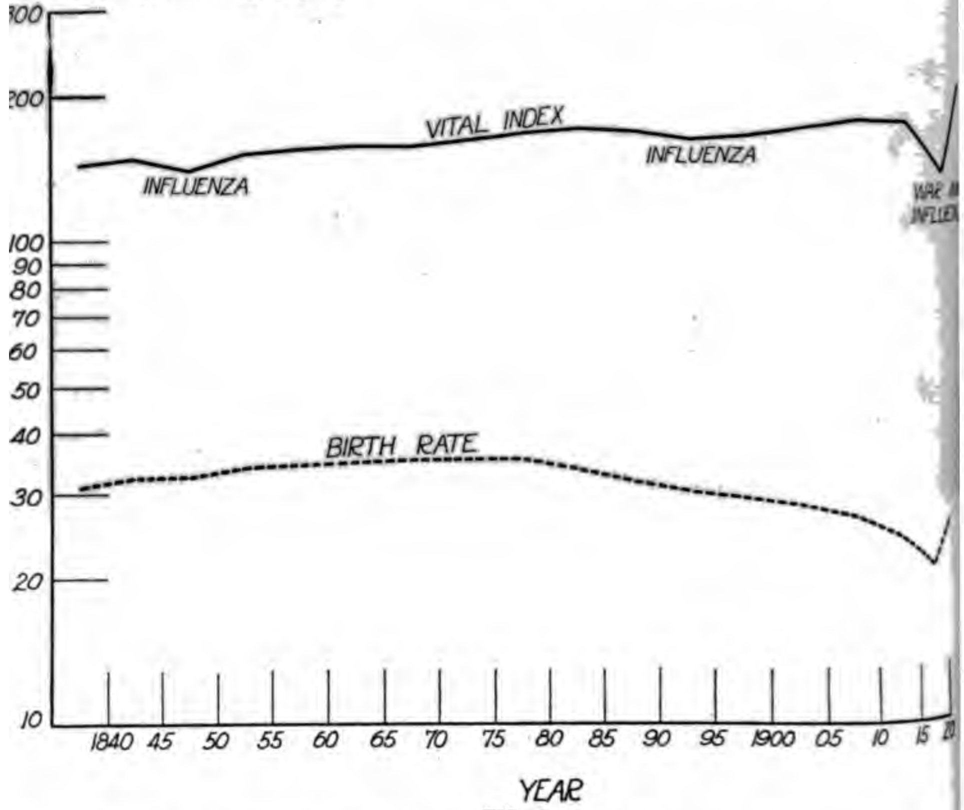

FIG. 1

Trend of vital index (100 births/deaths) and crude birth rate in England and Wales, 1838–1920, inc

The vital index and birth rate data from table 2 are shown graphically in figure 1.

The diagram is plotted on an arithlog grid, in order that the slopes of the lines may be strictly comparable.[6]

It is at once apparent that *the ratio of births to deaths in England and Wales had a slow but extremely even and steady increase from 1838 to 1914.* This steady progress was interrupted to a degree sufficient to be apparent upon only two occasions during the three quarters of a century. These were in 1847–1849 and 1890–1892. These fluctuations, which only slightly affected the even upward trend of the curve were due to the influenza pandemics of 1847–8 and 1890–1891. The broad result is perfectly clear and outstanding. The population of England and Wales is today biologically fitter and possessed of greater purely biological survival value as a whole population than it was three quarters of a century ago. Whether it is a mentally, morally or anthropmetrically fitter population does not now concern us. We are dealing at the moment solely with the fact that, taking the people of England and Wales as a whole, slightly over 2 babies were born for every death in a year in 1920, as against 1.4 babies per death in a year in 1838–1839. The question of a differential fertility among different social classes we shall discuss farther on in the paper.

Now this result, which is beyond any question a fact, will strike anyone informed as to the sociological and eugenical literature of the last two decades as curiously at variance with the pessimistic tenor of that literature, taken as a whole. It has been pronounced from high places that the general trend of the British people was biologically downwards, that they were in fact becoming biologically dangerously near to a decadent race. Abundant quotations in support of this contention could be cited, were space available and were it necessary. This gloomy view has had its foundation solely upon the fact that, since the quinquennium 1875–1880, the birth rate in England and Wales has been falling rather rapidly, as is clearly shown in figure 1. This fact has been brought out by Elderton[7] in great detail.

But from a purely biological view-point, what matters a falling birth rate if the death rate falls even more rapidly, so that the net survivorship at any instant of time is constantly getting higher? To this it will, of course, be answered at once by those who view with alarm the declining birth rate that the real crux of the matter is in the differential change in fertility. Nowadays the "best" people do not produce their due share of progeny, while the "worse" people over-produce. If one accepts the man-made definitions of "best" and "worst" this is plainly true for some civilizations at least. In the American population, however, as one of us[8] has recently shown, the element probably least desirable racially, the negro, has the lowest survival value as a group (vital index generally less than 100). But is old Dame Nature ever really interested in any test of "best" and "worst" *except survival?* There is no evidence as yet that she is. Measured by this test the population as a whole of England and Wales is today biologically more vigorous than it was in 1838. This is a plain *fact.* Whether this fact is, sociologically or eugenically considered, a bad thing or a good thing, is something more than a purely biological problem, and as such we are content to leave it to the discussion of others.

What seems to us of considerable interest biologically, is the extraordinary perfection, demonstrated in the figures here presented, of the self-regulation of population growth. Disturbances of the course of the birth rate have been compensated for, in the population and period under review, with the greatest nicety. It will be of interest to examine the facts for other populations, and this we hope later to do.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 50.

[2] R. Pearl, "The Effect of the War on the Chief Factors of Population Change," *Science* (N. S.), **51**, 1920 (553–556).

[3] Pearl, "The Vitality of the Peoples of America," *Amer. J. Hyg.*, **1**, 1921, pp. 592–674.

[4] Pearl, "A Further Note on War and Population," *Science* (N. S.), **53**, 1921, pp. 120–121.

[5] *Quarterly Return of Marriages, Births, and Deaths Registered in England and Wales, etc.* Published by Authority of the Registrar-General. London. H. M. Stationery Office. *Passim.* Also corresponding Weekly Return, and Annual Reports.

[6] "For a Discussion of the Significance and Advantages of Plotting Trend Lines on a Logarithmic Scale," cf. Fisher, I. "The 'ratio' Chart for Plotting Statistics," *Q. P. Am. Stat. Ass.*, **15**, 1917, pp. 577–601, and Field, J. A., "Some Advantages of the Logarithmic Scale in Statistical Diagrams," *J. Pol. Econ.*, **25**, 1917, pp. 805–841.

[7] E. M. Elderton, "Report on the English Birth Rate. Part I. England North of the Humber." *Eugenics Lab. Mem.*, 19 and 20, Cambridge Univ. Press, 1914, pp. viii and 246.

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ATHEMATICS.—*On the correlation between any two functions and its application to the general case of spurious correlation.*[1] LOWELL J. REED, Johns Hopkins University. (Communicated by Raymond Pearl.)

In problems where correlation methods are employed it is often ecessary to determine the coefficient of correlation between one of he measured variables and some function of the others. In certain ases we need to go still further and determine the correlation between wo different functions of the measured variables. Such cases arise here two different index numbers are correlated with each other r when an index number is correlated with one of the measured ariables. In all problems of this type the coefficient of correlation ay be found by computing the value of the function in question t each position for which the values of the variables themselves re known and then finding the correlation coefficient in the usual ay. In a great many cases however there would be a considerable ving of labor if this coefficient could be determined directly from e means, standard deviations, and first order correlation coefficients the variables themselves. The following general equation has en derived to accomplish this and it should prove to be of use in oblems of the type outlined above. The proof of the formula too long to be given in the present paper, but will be published ter, together with additional illustrations of its application.

t $x_1, x_2, \ldots\ldots\ldots\ldots x_n$ be a set of n variables,
d $x'_1, x'_2, \ldots\ldots\ldots\ldots x'_k$ be a second set of k variables.
et $m_1, m_2, \ldots\ldots\ldots m_n$ be the means of the variables of the first set,
$\sigma_1, \sigma_2, \ldots\ldots\ldots\ldots \sigma_n$ be their standard deviations,
d $r_{x_1x_2}, r_{x_1x_3} \ldots\ldots\ldots r_{x_{n-1}\,x_n}$ be the coefficients of correlation between ese variables taken in pairs.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene, hns Hopkins University, No. 38. Received October 18, 1921.

The same notation will be used for the means, standard deviations and coefficients of correlation of the primed variables.

Now let $y = f(x_1, x_2, \ldots\ldots x_n)$

and $z = f(x'_1, x'_2, \ldots\ldots x'_k)$

represent any two analytic functions of these two sets of variables. Then the correlation between these two functions is measured by

$$r_{yz} = \frac{\sum_{\alpha=1}^{n}\sum_{\beta=1}^{k} f_\alpha F_\beta \, r_{x_\alpha x_\beta}\sigma_\alpha\sigma_\beta}{\sqrt{\left[\sum_{\alpha=1}^{n}\sum_{\beta=1}^{n} f_\alpha f_\beta \, r_{x_\alpha x_\beta}\sigma_\alpha\sigma_\beta\right]\left[\sum_{\alpha=1}^{k}\sum_{\beta=1}^{k} F_\alpha F_\beta r_{x'_\alpha x'_\beta}\sigma'_\alpha\sigma'_\beta\right]}} \quad (1)$$

in which

$$f_\alpha = \frac{\partial f(m_1, m_2, \ldots\ldots m_n)}{\partial m_\alpha}$$

and

$$F_\beta = \frac{\partial F(m'_1, m'_2, \ldots\ldots m'_k)}{\partial m'_\beta}.$$

The derivation of equation (1) yielded as a by-product the expressions for the mean and standard deviation of any function of a set of variables in terms of the means, standard deviations and first order coefficients of correlation of the variables themselves.[2] These formulae are

(a) for the mean value of $y = f(x_1, x_2, \ldots\ldots\ldots x_n)$,

$$m_y = f + \tfrac{1}{2}\sum_{\alpha=1}^{n}\sum_{\beta=1}^{n} f_{\alpha\beta} \, r_{x_\alpha x_\beta}\sigma_\alpha\sigma_\beta \quad (2)$$

where $f = f(m_1, m_2, \ldots\ldots\ldots\ldots m_n)$

and $$f_{\alpha\beta} = \frac{\partial^2 f(m_1, m_2, \ldots\ldots m_n)}{\partial m_\alpha \partial m_\beta}$$

(b) for the standard deviation of $y = f(x_1, x_2, \ldots\ldots x_n)$,

$$\sigma_y = \sqrt{\sum_{\alpha=1}^{n}\sum_{\beta=1}^{n} f_\alpha f_\beta \, r_{x_\alpha x_\beta}\sigma_\alpha\sigma_\beta} \quad (3)$$

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[2] For another derivation of equations (2) and (3) see E. CZUBER, *Über Funktionen von Variablen zwischen welchen Korrelationen bestehen.* Metron **1**, No. 1, July, 1920.

Equations (1), (2) and (3) furnish a complete set of formulae for the determination of the means, standard deviations, and coefficient of correlation of any two functions of two sets of variables in terms of the means, standard deviations, and coefficients of correlation of the variables themselves.

It should be noted that, in the derivation of these formulae, all terms of the third or higher orders in the variables $\frac{\sigma_1}{m_1}, \frac{\sigma_2}{m_2}$, etc., were disregarded. Therefore in a practical problem when the standard deviation is large as compared with the mean the formula might not give a sufficiently close approximation to the true value of the correlation coefficient. In the majority of cases, however, the ratio of the standard deviation to the mean is a sufficiently small decimal so that disregarding its powers higher than the second can have no appreciable effect on the result.

To illustrate the use of equation (1) we may first apply it to the case of the correlation between ratios. Let the variables be x_1, x_2 and x_1', x_2' and the ratios be $y = \frac{x_1}{x_2}$ and $z = \frac{x_1'}{x_2'}$.

Then $$f_1 = \frac{1}{m_2},\ f_2 = -\frac{m_1}{m_2{}^2}$$

$$F_1 = \frac{1}{m_2'},\ F_2 = -\frac{m_1'}{m_2'^2}.$$

Substituting these values in (1) and replacing $\frac{\sigma_1}{m_1}$ by v_1, $\frac{\sigma_2}{m_2}$ by v_2, etc., we have

$$r_{yz} = \frac{r_{x_1x_1'}v_1v_1 - r_{x_1x_2'}v_1v_2 - r_{x_2x_1'}v_2v_1' + r_{x_2x_2'}v_2v_2'}{\sqrt{(v_1{}^2 + v_2{}^2 - 2r_{x_1x_2}v_1v_2)(v_1{}^2 + v_2'^2 - 2r_{x_1'x_2'}v_1'v_2')}}. \qquad (4)$$

This is Pearson's[3] well known formula for the coefficient of correlation of two indices or ratios. Formulae for the means and standard deviations of y and z can be obtained from equations (2) and (3).

They are $$m = \frac{m_1}{m_2}\left[1 + v_2{}^2 - r_{x_1x_2}v_1v_2\right]$$

and $$\sigma_y = \frac{m_2}{m_1}\sqrt{v_1{}^2 + v_2{}^2 - 2r_{x_1x_2}v_1v_2}.$$

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[3] PEARSON, K. *On a form of spurious correlation which may arise when indices are used in the measurement of organs.* Proc. Roy. Soc., London 60. 1896.

As a second illustration let us consider the correlation between products of the variables.

Let $y = x_1x_2$ and $z = x_1'x_2'$.

Then from (1) we have

$$r_{yz} = \frac{r_{x_1x_1'}v_1v_1' + r_{x_2x_1'}v_2v_1' + r_{x_1x_2'}v_1v_2' + r_{x_2x_2'}v_2v_2'}{\sqrt{(v_1^2 + v_2^2 + 2r_{x_1x_2}v_1v_2)\,(v_1'^2 + v_2'^2 + 2r_{x_1'x_2'}v_1'v_2')}}. \tag{5}$$

The mean and standard deviation, derived from equations (2) and (3), are given by

$$m_y = m_1m_2\,[1 + r_{x_1x_2}v_1v_2]$$

and

$$\sigma_y = m_1m_2\sqrt{v_1^2 + v_2^2 + 2r_{x_1x_2}v_1v_2}.$$

If we consider x_2' to be constant we have a special case of formula (5) of considerable importance; that is, the case of the correlation between the product of two variables, $y = x_1x_2$, and some third variable $z = x_1'$.

For this case

$$r_{yz} = \frac{r_{x_1x_1'}v_1 + r_{x_2x_1'}v_2}{\sqrt{v_1^2 + v_2^2 + 2r_{x_1x_2}v_1v_2}}. \tag{6}$$

Formulae (5) and (6) will be found useful in those cases in which the product of two measurable linear functions is used as an index number for a surface that cannot be directly measured, as is the case for instance in dealing with the surface of the human body.

The problem of finding the coefficient of correlation between some function of a set of variables and some other measured variable is so common that it is advisable to consider the form taken by equation (1) in this case. We would have

$$y = f(x_1, x_2, \ldots\ldots\ldots\ldots x_n) \text{ and } z = F(x_1') = x_1'.$$

Then

$$r_{yz} = \frac{\sum_{\alpha=1}^{n} f_\alpha r_{x_\alpha x_1'}\sigma_\alpha.}{\sqrt{\sum_{\alpha=1}^{n}\sum_{\beta=1}^{n} f_\alpha f_\beta r_{x_\alpha}\sigma_\alpha\sigma_\beta}}. \tag{7}$$

It should be noted that the standard deviation of the variable x_1' does not enter this equation directly, the only factor involving x_1' being the correlation coefficient $r_{x_\alpha x_1'}$. Equation (6) which was derived as a special case of formula (5) might have been obtained directly from (7).

SPURIOUS CORRELATION

Attention was first called to the subject of spurious correlation in a paper by Pearson[4] where he considered the case of the spurious correlation between two ratios of the form $\frac{x_1}{x_3}$ and $\frac{x_2}{x_3}$. Pearson showed that although x_1, x_2, and x_3 were uncorrelated variables there would be correlation between the two ratios $\frac{x_1}{x_3}$ and $\frac{x_2}{x_3}$, this correlation arising from the fact that x_3 is common to the two indices. Since this correlation exists where there is no correlation between x_1, x_2 and x_3 he gave it the name of *spurious correlation*. The arguments used by Pearson in connection with the spurious correlation between ratios will hold in the case of correlation between any two functions, and a general definition of spurious correlation might be given as follows.

Though no correlation exists between any two of a set of variables there will still exist correlation between any two functions of these variables whenever these two functions have any of the variables in common. The correlation existing under these conditions will be called *spurious correlation*.

A general formula for spurious correlation may be derived directly from equation (1). Using, as before, $y = f(x_1, x_2, \ldots\ldots x_n)$ and $z = F(x_1', x_2' \ldots\ldots\ldots x_{k'})$ we shall have spurious correlation only when some of the variables $x_1, x_2, \ldots\ldots x_n$ are identical with some of the variables $x_1', x_2' \ldots\ldots x_{k'}$.

Let $x_1 = x_1'$
$x_2 = x_2'$
$(\quad \cdots \quad)$
$x_h = x_h'$ where $h \overline{<} k$ and $h \overline{<} n$.

Then, from the definition of spurious correlation, $r_{x_\alpha x_\beta} = 0$ except for the cases where $\alpha = \beta \overline{<} h$ in which event $r_{x_\alpha x_\beta} = 1$.

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[4] *Op. cit.*

Thus we shall have

$$\rho_{yz} = \frac{\sum_{\alpha=1}^{h} f_\alpha F_\alpha \sigma_\alpha^2}{\sqrt{\left[\sum_{\alpha=1}^{n} f_\alpha^2 \sigma_\alpha^2\right]\left[\sum_{\alpha=1}^{k} F_\alpha^2 \sigma_\alpha'^2\right]}} \qquad (8$$

where ρ is used to denote spurious correlation.

All of the special cases considered under equation (1) will exhibit or more forms of spurious correlation.

For example, when $y = \frac{x_1}{x_2}$ and $z = \frac{x_1'}{x_2'}$ we may have three diffe cases of spurious correlation:

(a) when the fractions have the same denominator, *i. e.*, $x_2 = x_2'$

(b) when the denominator of one is the same as the numerator of other, *i. e.*, $x_2 = x_1'$

(c) when the fractions have common numerators, *i. e.*, $x_1 = x_1'$.

The formulae for the spurious correlation in these three cases are

(a) $$\rho_{yz} = \frac{v_2^2}{\sqrt{(v_1^2 + v_2^2)(v_1'^2 + v_2^2)}} \qquad (9$$

(b) $$\rho_{yz} = \frac{-v_2^2}{\sqrt{(v_1^2 + v_2^2)(v_2^2 + v_2'^2)}} \qquad (10$$

(c) $$\rho_{yz} = \frac{v_1^2}{\sqrt{(v_1^2 + v_2^2)(v_1^2 + v_2'^2)}}. \qquad (1$$

Of these three cases the first one is the one which usually arise practice.

When the functions considered are products they may hav common factor so that $y = x_1x_2$ and $z = x_1'x_2$.

Then

$$\rho_{yz} = \frac{v_2^2}{\sqrt{(v_1^2 + v_2^2)(v_1'^2 + v_2^2)}}. \qquad (12$$

The foregoing illustrations show a few of the equations for specific cases of spurious correlation that can be derived from the general formula. Similar expressions may be obtained from equation (8) for the spurious correlation between any two functions. The value of the spurious correlation involved should always be considered when drawing conclusions from the coefficient of correlation of any two index numbers regardless of their functional form.

Fi [illegible]

The problem may be stated as follows: Given a set of poi $P_1, P_2, — P_n$ whose coordinates are (x_1y_1), (x_2y_2), — (x_ny_n), find a straight line so situated that the sum of the squares the normal deviations of the points from the line will be a nimum. Using the equation of the line in the form

$$(1) \qquad x\cos\alpha + y\sin\alpha = p$$

the value of the residual for the point P_1 is given by

$$r_1 = x_1\cos\alpha + y_1\sin\alpha - p$$

This residual r_1 is the line P_1L_1 of Figure 1. Now the sum the squares of the residuals for all the points will be

$$(2) \qquad \begin{aligned} \Sigma r^2 = {} & \cos^2\alpha\Sigma x^2 + \sin^2\alpha\Sigma y^2 + n^2p^2 \\ & + 2\sin\alpha\cos\alpha\Sigma xy - 2p\cos\alpha\Sigma x - 2p\sin\alpha\Sigma y \end{aligned}$$

To make this function a minimum its partial derivatives wi respect to p and α must vanish so that we have

$$(3) \qquad \begin{aligned} \frac{\partial\Sigma r^2}{\partial p} &= 2np - 2\cos\alpha\Sigma x - 2\sin\alpha\Sigma y = 0 \\ \text{or} \qquad & \cos\alpha\Sigma x + \sin\alpha\Sigma y - np = 0 \end{aligned}$$

and

$$(4) \qquad \begin{aligned} \frac{\partial\Sigma r^2}{\partial\alpha} = {} & -2\sin\alpha\cos\alpha\Sigma x^2 + 2\sin\alpha\cos\alpha\Sigma y^2 + 2(\cos^2\alpha - \sin^2\alpha\Sigma \\ & + 2p\sin\alpha\Sigma x - 2p\cos\alpha\Sigma y = 0 \end{aligned}$$

Dividing (3) by n we have

$$\cos\alpha\frac{\Sigma x}{n} + \sin\alpha\frac{\Sigma y}{n} - p = 0$$

which shows that the coordinates of the center of gravity the set of points satisfy equation (1) and the line must ther pass through the center of gravity. If now we assume the or at this point and let $\bar{x}$, $\bar{y}$ be the coordinates of any point refer to this origin, equation (4) takes the form

$$-\sin 2\alpha\,\Sigma\bar{x}^2 + \sin 2\alpha\,\Sigma\bar{y}^2 + 2\cos 2\alpha\,\Sigma\bar{x}\bar{y} = 0$$

which gives

(5)
$$\tan 2\alpha = \frac{2\,\Sigma \bar{x}\bar{y}}{\Sigma \bar{x}^2 - \Sigma \bar{y}^2}$$

Now equation (5) is satisfied by two values of α that differ from each other by 90° so that there are two lines passing through the center of gravity at right angles to each other that satisfy conditions (3) and (4). Applying the usual tests we find that one line makes the sum of the squares of the residuals a minimum and the other makes it a maximum. One may, therefore, be called the best fitting straight line and the other the poorest fitting straight line. Referring to Figure 1 the best fitting straight line is LM and the poorest is PQ. The usual calculus tests may be applied to determine which value of α gives the minimum and which the maximum, but this is hardly necessary in a practical problem.

A mechanical illustration of this type of fit can be given as follows: Suppose the line LM of Figure 1 to be a fine steel rod lying on a frictionless plane, and let the rod to attached to each of the points P_1, P_2, — P_n by elastic bands, the bands being so attached to the rod that there is no friction between band and rod so that the elastic always maintains a position perpendicular to the rod. Then if the equation of the position of the rod be given by

$$x \cos \alpha + y \sin \alpha = p$$

the force with which the elastic at any point P_β pulls the rod is given by

$$F_\beta = K(x_\beta \cos \alpha + y_\beta \sin \alpha - p)$$

where K is a constant.

Then to find the position of equilibrium of the rod we have

(6)
$$\Sigma F = K(\cos \alpha \Sigma x + \sin \alpha \Sigma y - p) = 0$$

since the sum of the forces normal to the rod must vanish. Also, since the sum of the moments of the forces about the origin must be zero we have

$$\Sigma M = \Sigma K(x \sin \alpha - y \cos \alpha)\ (x \cos \alpha + y \sin \alpha - p) = 0$$

or

(7)
$$K\Big[\sin \alpha \cos \alpha\, \Sigma x^2 + (\sin^2 \alpha - \cos^2 \alpha)\ \Sigma xy - \sin \alpha \cos \alpha \Sigma y^2 \\ - p \sin \alpha \Sigma x + p \cos \alpha \Sigma y\Big] = 0$$

These two equations (6) and (7) allow us to solve for th values of a and p that represent the position of equilibrium o the rod. But these equations are the same equations as (3) and (4 therefore the positions of equilibrium are the same as the pc sitions of the best and poorest fitting straight lines. The bes fitting straight line is a position of stable equilibrium of the roc Thus, when we fit a straight line by making the sum of th squares of the normal residuals a minimum we have the sam result as if we allowed the line to come to rest with each poin exerting a normal pull on the rod that is proportional to th distance of the point from the line.

To illustrate the use of formula (5) let us fit a straight lin to the death rate from all causes in Italy from 1880 to 1910 The annual death rates per thousand averaged over five yea periods were (1).

1881-1885 = 27.3	1896-1900 = 22.9
1886-1890 = 27.2	1901-1905 = 21.9
1891-1895 = 25.2	1906-1910 = 21.0

Taking the origin at 1880.5 the coordinates of the points t which we are to fit a straight line are given in columns x an y in the table below.

x	y	x^2	y^2	xy
2	27.3	4	745.29	54.6
7	27.2	49	739.84	190.4
12	25.2	144	635.04	302.4
17	22.9	289	524.41	389.3
22	21.9	484	479.61	481.8
27	21.0	729	441.00	567.0
87	145.5	1699	3565.19	1985.5

Now the coordinates of the center of gravity are

$$\frac{\Sigma x}{n} = 14.5 \quad \text{and} \quad \frac{\Sigma y}{n} = 24.25$$

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(1) See Bureau of the Census, *U. S. Mortality Statistics, 1910*, p. [illegible]

The problem may be stated as follows: Given a set of p P_1, P_2, — P_n whose coordinates are $(x_1 y_1)$, $(x_2 y_2)$, — $(x_n y_n$ find a straight line so situated that the sum of the squar the normal deviations of the points from the line will be a nimum. Using the equation of the line in the form

$$(1) \qquad x \cos \alpha + y \sin \alpha = p$$

the value of the residual for the point P_1 is given by

$$r_1 = x_1 \cos \alpha + y_1 \sin \alpha - p$$

This residual r_1 is the line $P_1 L_1$ of Figure 1. Now the sum the squares of the residuals for all the points will be

$$(2) \qquad \begin{aligned} \Sigma r^2 = {} & \cos^2 \alpha \Sigma x^2 + \sin^2 \alpha \Sigma y^2 + n^2 p^2 \\ & + 2 \sin \alpha \cos \alpha \Sigma xy - 2p \cos \alpha \Sigma x - 2p \sin \alpha \Sigma y \end{aligned}$$

To make this function a minimum its partial derivatives wi respect to p and α must vanish so that we have

$$(3) \qquad \begin{aligned} \frac{\partial \Sigma r^2}{\partial p} &= 2np - 2 \cos \alpha \Sigma x - 2 \sin \alpha \Sigma y = 0 \\ \text{or} \qquad & \cos \alpha \Sigma x + \sin \alpha \Sigma y - np = 0 \end{aligned}$$

and

$$(4) \qquad \begin{aligned} \frac{\partial \Sigma r^2}{\partial \alpha} = {} & - 2 \sin \alpha \cos \alpha \Sigma x^2 + 2 \sin \alpha \cos \alpha \Sigma y^2 + 2 (\cos^2 \alpha - \sin^2 \alpha \\ & + 2p \sin \alpha \Sigma x - 2p \cos \alpha \Sigma y = 0 \end{aligned}$$

Dividing (3) by n we have

$$\cos \alpha \frac{\Sigma x}{n} + \sin \alpha \frac{\Sigma y}{n} - p = 0$$

which shows that the coordinates of the center of gravity the set of points satisfy equation (1) and the line must theref pass through the center of gravity. If now we assume the orig at this point and let x, y be the coordinates of any point refer to this origin, equation (4) takes the form

$$- \sin 2\alpha \Sigma \bar{x}^2 + \sin 2\alpha \Sigma \bar{y}^2 + 2 \cos 2\alpha \Sigma \bar{x} \bar{y} = 0$$

METRON

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THE PROBABLE ERROR OF THE VITAL INDEX OF A POPULATION[1]

BY JOHN RICE MINER

DEPARTMENT OF BIOMETRY AND VITAL STATISTICS, JOHNS HOPKINS UNIVERSITY

Communicated February 14, 1922

In comparing the birth-death ratios of different communities we need their probable errors to determine the significance of observed differences.

Let N = the population of a community,
B = number of births in a year,
D = number of deaths in a year.

Then the birth-death ratio or vital index is defined as $\frac{100\,B}{D}$.

The standard errors of sampling of births and deaths will be:

$$\sigma_B = \sqrt{\frac{B(N-B)}{N}} \qquad \sigma_D = \sqrt{\frac{D(N-D)}{N}}$$

whence $V_B = \frac{\sigma_B}{B} = \sqrt{\frac{1 - B/N}{B}}$ $\qquad V_D = \frac{\sigma_D}{D} = \sqrt{\frac{1 - D/N}{D}}$

and the probable error of the birth-death ratio will be[2]

$$PE_{\left\{\frac{100\,B}{D}\right\}} = 67.449\frac{B}{D}\sqrt{\frac{1 - B/N}{B} + \frac{1 - D/N}{D}} \qquad \text{(i)}$$

since $r = 0$, being the correlation of errors of sampling of births and death As B and D are small as compared with N we may write this approximatel

$$PE_{\left\{\frac{100\,B}{D}\right\}} = 67.449\frac{B}{D}\sqrt{\frac{1}{B} + \frac{1}{D}} \qquad \text{(ii)}$$

This form has the advantage of not involving N, so that if the numbe of births and deaths in a community are known but not the populatio the probable error of the birth-death ratio may still be found.

For Baltimore in 1917

$N = 594{,}637$
$B = 15{,}040$
$D = 11{,}355.$

Therefore by (i)

$$PE_{\left\{\frac{100\,B}{D}\right\}} = 67.449\,\frac{15040}{11355}\sqrt{\frac{1 - \frac{15040}{594637}}{15040} + \frac{1 - \frac{11355}{594637}}{11355}}$$

$$= 89.34\sqrt{\frac{0.9747}{15040} + \frac{0.9809}{11355}} = 89.34\ \sqrt{.00006481 + .00008638}$$

$$= 89.34 \times .01229 = 1.10.$$

By (ii)

$$PE_{\left\{\frac{100\,B}{D}\right\}} = 67.449\,\frac{15040}{11355}\ \sqrt{\frac{1}{15040} + \frac{1}{11355}} = 89.34\ \sqrt{.00006649 + .00008807}$$

$$= 89.34 \times .01243 = 1.11.$$

In using these formulae it should be borne in mind that the expressio for the standard deviation of an index, which has been used in their der ivation, is obtained on the assumption that deviations are small compare with the mean values of the variables. This will be true if B and D are o fair size, but the formulae should not be used for very small absolute numbers of births and deaths.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 52.

[2] Pearson, K. On a form of spurious correlation which may arise when indices are used in the measurement of organs. *Proc. Roy. Soc.*, **60**, 1897 (489).

NOTE ON A CASE OF HUMAN INBREEDING

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NOTE ON A CASE OF HUMAN INBREEDING[1]

Through the kindness of a friend the following pedigree is presented. It is that of a family of English stock, which has been in this country since the early eighteenth century, and during that time has been one of the principal families of a rural community.

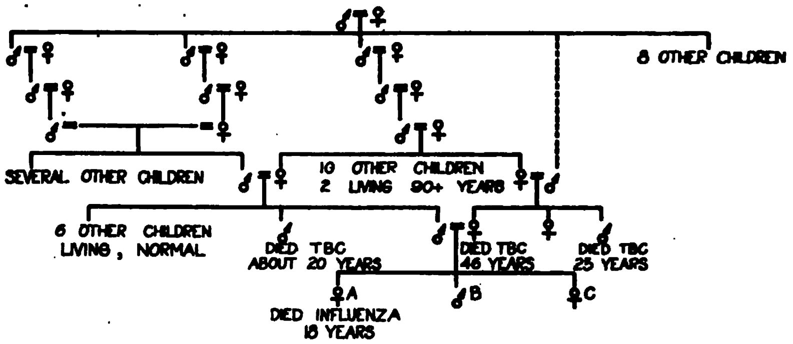

Fig. 1. Pedigree of an inbred family.

To quote from my correspondent's letter, "A was a fine young girl. She had graduated from high school but did not go to college as her mother had died in the summer and she wished to take charge of the home. B is about 16 years old. A splendid young man, bright and apparently healthy. He is in school standing about average. C is 10 years old or thereabouts. An exceptionally bright child and one that is very much alive and full of spirit."

Assuming that the line of descent represented in the figure by a broken line, indicating that the number of generations is not known, includes the same number of generations as the other lines the coefficients of inbreeding[2] for the children in the last generation (viz., A, B, and C) are as follows:

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 41.

[2] Pearl, "Studies on Inbreeding," I–VIII, American Naturalist, 1913–17.

$$Z_1 = 0, \qquad Z_4 = 25,$$
$$Z_2 = 25, \qquad Z_5 = 34.37,$$
$$Z_3 = 25, \qquad Z_{T_5} = 27.1,$$

i.e., in five generations of ancestry the inbreeding is about a quarter of the possible maximum.

There is no deleterious effect of inbreeding apparent in this pedigree. The three children in the last generation, the most inbred of any, show no signs of abnormality. In their father's fraternity, for which $Z_1 = Z_2 = Z_3 = 0$; $Z_4 = 12.5$; $Z_{T_4} = 4.1$, or in four generations of ancestry there is $\frac{1}{25}$ of the possible maximum inbreeding, one of eight died of tuberculosis; the other seven have attained adult age. In the mother's fraternity, for which $Z_1 = Z_2 = Z_3 = 0$; $Z_4 = 6.25$; $Z_{T_4} = 2.0$, two out of the three have died of tuberculosis. The least inbred, therefore, show the greatest susceptibility to tuberculosis. The numbers are, of course, too small to draw any certain inference, but so far as they go, they accord best with the view that there is no harmful effect of inbreeding per se.

John Rice Miner

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*NOTE ON MOVING EQUILIBRA**

BY ALFRED J. LOTKA
BROOKLYN, N. Y.

Communicated by R. Pearl, March 12, 1921

A number of previous publications[1] have been devoted to the study, from various angles, of a material system evolving in accordance with a system of differential equations

$$dX_i \, / \, dt = F_i(X_1, X_2, \ldots; A_1, A_2, \ldots; P; Q) \qquad (1)$$

where the X's denote the masses of the several components of which the system is built up; the A's are parameters introduced by any equations of constraint to which the X's may be subject;[2] the parameters P include geometrical constraints (volume, area, topography) and also other quantities serving to define the state of the system (temperature, etc.). The Q's define the character of the several components or species.

The discussion has hitherto been restricted to the case that the parameters A, P, Q remained constant during the transformations taken in view. A complete discussion of the evolution of systems of the kind referred to must include also the consideration of changes in these parameters.

Such changes may be grouped under three heads:

1. Changes of a perfectly general character. A study of these would resolve itself into a discussion, on a general basis, of a system of differential equations of the form

$$dX_i \, / \, dt = F_i(X_1, X_2, \ldots, t) \qquad (2)$$

It is not intended to enter here into this perfectly general case. It will suffice to point to the purely mathematical literature on the subject.[3]

2. Changes in the parameters A, P, Q proceeding very slowly as compared with the speed of readjustment of the X's. In this case the system, after passing through a "transient" state, ultimately settles down to a "moving equilibrium." The study of this case therefore comprises two phases, which may with advantage be taken up separately. The phase of the moving equilibrium is of particular interest, since such moving equilibria play an important rôle in the evolution of physical systems, as pointed out years ago by Herbert Spencer.[4]

3. We may study the effect of a change in a parameter A, P or Q upon the equilibrium of the system alone, irrespective of the process by which that equilibrium is reached. It is in this case immaterial whether the change is slow or rapid. Into this division of the subject fall such relation as the principle of Le Chatelier, the thermodynamic laws of equilibrium and the "reciprocal relations" of statistical mechanics.

The application of some of these principles to biological and social systems has been essayed. but it cannot be said that the rigor of the attempts thus made is satisfactory. It would therefore be desirable to go over the ground and consolidate it. An effort in this direction is taken in view as part of the plan into which the present contribution is fitted.[5]

Of the general field outlined above, the portion to which we shall now give our attention is that of moving equilibria.

Our fundamental system of equations we shall, for our present purpose, write in the form (2). Furthermore, merely in order to simplify our notation, we will restrict the number of dependent variables to two, which, to avoid subscripts, we will denote by X, Y. We have, then

$$dX/dt = F_1(X, Y, t), \qquad dY/dt = F_2(X, Y, t). \tag{3}$$

We adopt a method of successive approximations. Since the system is near equilibrium, we write for our first approximation

$$0 = F_1(X, Y, t), \qquad 0 = F_2(X, Y, t). \tag{4}$$

Solving for X and Y we then have

$$X_1 = \varphi_1(t), \qquad X_1 = \psi_1(t). \tag{5}$$

whence by differentiation

$$dX_1/dt = X_1' = \varphi'_1(t), \qquad dY_1/dt = Y'_1 = \psi'_1(t). \tag{6}$$

Proceeding to a second approximation, we substitute (6) in (3).

$$\varphi'_1(t) = F_1, \qquad \psi'_1(t) = F_2. \tag{7}$$

Solving again for X, Y,

$$X_2 = \varphi_2(t), \qquad Y_2 = \psi_2(t.) \tag{8}$$

We may again differentiate,

$$X'_2 = \varphi'_2(t), \qquad Y_2 = \psi'_2(t). \tag{9}$$

and, substituting as before, we obtain a third approximation. We continue this process as far as may be desired, and finally obtain for the n *th* approximation

$$X_n = \varphi_n(t), \qquad Y_n = \psi_n(t). \tag{10}$$

If the functions φ_n, ψ_n, and φ'_n, ψ'_n, obtained by these successive approximations tend toward a limit as n is increased indefinitely, then it can easily be shown that (10) is a solution of (3).

It seems to be somewhat difficult to give in general terms the conditions for this convergence of successive approximations toward a limit. However, the following example will show how in individual cases the question regarding this convergence can sometimes be readily answered.

For our example we take the case of radioactive equilibrium. We have a chain of transformations

$$A \longrightarrow B \longrightarrow C \longrightarrow D \tag{11}$$

We will denote the masses of A, B, C, D at time t, respectively, by U, X, Y, Z, and their values at time $t=0$ by the same letters with the subscript zero.

We may if we choose (this is a purely arbitrary matter) pick out the substances B and C for our evolving system, and look upon A and D as external factors influencing the system. The system composed of B and C is then subject to an equation of constraint

$$X + Y = X_0 + Y_0 + U_0 - U + Z_0 - Z \tag{12}$$

in which the quantities appearing in the right hand member are parameters of the class denoted above in equation (1) by A, that is to say, parameters introduced by the equation of constraint. Four of them are constants, the other two are functions of t. Of these last two one, namely U, will appear in the equations representing the course of evolution of the system composed of B and C. These equations, according to the well-known laws of radioactive transformations are

$$dX/dt = aU - bX, \qquad dY/dt = bX - cY. \tag{13}$$

where a, b, c are constants. U, on the other hand, is a function of the time, namely

$$U = U_0e^{-at} \tag{14}$$

For our first approximation we put, then,

$$dX/dt = 0 = aU_0e^{-at} - bX_1 \qquad dY/dt = 0 = bX_1 - cY_1 \tag{15}$$

whence

$$X_1 = a/b.\ U_0e^{-at}, \qquad Y_1 = a/c.\ U_0e^{-at}, \tag{16}$$

and therefore

$$X'_1 = -a^2/b.\ U_0e^{-at}, \qquad Y'_1 = -\ a^2/c.\ U_0e^{-at}. \tag{17}$$

The second approximation now gives

$$dX/dt = X'_1 = -a^2/b.\ U_0e^{-at} = aU_0e^{-at} - bX_2 \tag{18}$$

$$dY/dt - Y'_1 = -a^2/c.\ U_0e^{-at} = bX_2 - cY_2 \tag{19}$$

Whence

$$X_2 = a/b.\ U_0e^{-at}\ (1 + a/b) \tag{20}$$

$$Y_2 = a/c.\ U_0e^{-at}\ (1 + a/b + a/c) \tag{21}$$

Continuing this process we find, ultimately,

$$X_n = a/b\, U_0 e^{-at} \left(1 + a/b + a^2/b^2 + \ldots\right) \tag{22}$$

$$Y_n = a/c\, U_0 e^{-at} \left(1 + a/b + a/c + a^2/b^2 + a^2/bc + a^2/c^2 + \ldots\right) \tag{23}$$

In this example the condition for the convergence of the successive approximations is immediately apparent. We must have

$$a/b < 1, \qquad a/c < 1; \tag{24}$$

that is to say, the parent substance A must have a smaller decay constant than any of the succeeding members of the series. For obvious reasons this condition is always satisfied in natural radioactive mixtures.[6]

The series (22), (23) bring out the relation between the uncorrected equilibrium, as commonly computed on the assumption of constancy of mass of the parent substance, and the true equilibrium. The first-mentioned (for which Rutherford has suggested the term "secular equilibrium") is represented by the first term of the series. As Rutherford points out, the error of the first approximation, i.e. the difference between the secular and the true equilibrium, amounts, in some cases, to nearly 1% though in others the error is quite negligible.

The series are easily summed, and then lead to the well-known expressions obtained by other methods (for the equations of radioactive change are readily integrable in finite terms, while the method here developed is applicable also in more refractory cases).

The case of radioactive equilibrium was here selected as an illustration, primarily because the functions involved are known and of simple form. But the same example will serve very aptly to illustrate also some other points.

In the first place we observe that moving equilibria might be divided into three classes, according as their progress is determined by a change in the P's, the Q's or the A's. As has been shown, the radioactive equilibrium is of the type in which the pace is set by a parameter of the class A, namely *the mass of one of the links in the chain*, which thus acts as a brake, or a *limiting factor* checking the series of transformations. Such limiting factors play an important rôle also in the highly complex network of interlocking cycles upon which the continuance of abundant life upon the earth depends. For life processes are energy transformation processes carried out by the agency of material energy transformers. Such transformers, if they are to work continuously and indefinitely must perforce work in closed transformation chains or cycles (such as the cycle $CO_2 \longrightarrow$ Plant $\longrightarrow$ Animal $\longrightarrow CO_2$). The moving equilibria engendered in such systems of cycles by a slow change in a limiting factor, in a parameter of class A, invite further study. The influence of man upon the world's events seems to have been largely to accelerate the circulation of matter and energy through such cycles, either by "enlarging the wheel", i.e., increasing the mass taking part in certain cycles, or else by causing it to "spin faster," i.e., increasing the velocity of the circulation, decreasing the time required for a given mass to complete the cycle. In either case he has increased the energy turn-over per unit of time. Whether, in this he has been unconsciously fulfilling one of those laws of nature according to which certain quantities tend toward a maximum, is a question well deserving of our attention.

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[*] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 44.

[1] Lotka, A. J., *Physic. Rev.*, **24**, 1912 (235–238); *J. Washington Acad. Sci.*, **2**, 1912 (2, 49, 66); *Science Progress*, **55**, 1920 (406–417); *Proc. Am. Acad. Arts Sci.*, **55**, 1920 (237–153); these PROCEEDINGS, *Sci.* **6**, 1910 (410–415).

[2] *Proc. Amer. Acad.*, loc cit., p. 142.

[3] See for example Picard, *Traité d'Analyse;* H. Bateman, *Differential Equations,* 1918, p. 245.

[4] Spencer, *First Principles,* Chapter XXII; Winiarskie, "Essai sur la Mécanique Sociale," *Revue Philosophique,* **44,** 1900 (113).

[5] At the time of reading proof this project is partially realized. A discussion of the applicability of the Le Chatelier principle to systems of the general character here considered will appear in a forthcoming issue of the *Proceedings of the American Academy of Arts and Sciences*

[6] Lotka, A. J., *London, Phil. Mag.*, Aug., 1911, p. 353.

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NOTE ON THE ECONOMIC CONVERSION FACTORS OF ENERGY[1]

BY ALFRED J. LOTKA

BROOKLYN, NEW YORK

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That there is some relation between physical energy and economic value has been more or less clearly recognized by several authors. So G. Helm, for example, conceives of an "economic energy," whose capacity factor is quantity (of commodity), and whose intensity factor is measured by the price paid per unit quantity. A certain justification for this standpoint may be found in the fact that on the one hand goods having economic value can be exchanged for, and in this sense converted into energy; while on the other hand the movement of commodities in an economic system, that is to say, the transfer of property by sale and purchase, is determined by differences in economic value in much the same way as the transfer of energy from one portion of a physical system to another is determined by differences in the intensity factor of the energy concerned in the change.

From Helm's[2] standpoint, money would represent the most fluid form of economic energy, convertible freely into any other form of economic energy. Attention to this rôle of money is also drawn by Ostwald, who, however, expressly makes the reservation that money, although bearing a certain resemblance to energy, is not essentially identical therewith.[3]

Nevertheless, as we all know, money is convertible, by purchase upon the market, into various forms of energy, at rates somewhat variable, yet in some manner determinate.

The question therefore arises, what is it that determines the ratio of conversion of various forms of energy by exchange upon the market?

The physicist is familiar with two kinds of conversion factors of energy.

The first kind of factor is that which enters into the analytical expression of the first law of thermodynamics, the expression of the constancy in ratio of the amounts of several forms of energy replacing each other in physical transformations.

The second kind of factor relates to that fraction of energy transformed, which can be recovered in a selected form, and expresses the "efficiency" of the transformation or of the transformer taken in view.

It is one of the central data of thermodynamics that the equivalence factor is always independent of the mechanism by which the transformation is effected, while the efficiency factor is thus independent in the ideal case of a reversible transformation

It is this independence which makes the two laws of thermodynamics so fertile as tools for drawing conclusions regarding the course of physical events. Anyone who has ever sought to show a perpetual motion inventor just why his machine cannot work will appreciate the economy of thought and language which is secured by direct application of these principles.

Now in the transformation of energy by economic exchange upon the market we are dealing with a third type of conversion factor. The physical relations here involved are so complex that we are apt to overlook altogether that they *are* physical. Still less do we ordinarily recognize their precise character.

A simple example may help to clarify the view: the case of the automatic vending machine, the penny-in-the-slot chocolate dispenser, for instance.

The salient facts here are:

1. A definite amount of money brings in exchange a definite amount of commodity (and of energy).

2. The physical process is a typical case of "trigger action," in which the ratio of energy set free to energy applied is subject to no restricting general law whatever (e.g., a touch of the finger may set off tons of dynamite).

3. In contrast with the case of thermodynamic conversion factors, the

proportionality factor is here determined strictly by the particular mechanism employed.

Reflection shows that all transformations of money or of economic assets of any kind into energy by exchange upon the market are of this character. It is always a case of trigger action. Somewhere there is a store of available energy, which can be tapped with an expenditure of greater or less effort. The payment of the price sets in motion the requisite machinery for the release of that energy (or for its transfer of ownership, the release being delayed at the discretion of the buyer).

In view of the entire absence of any general law regulating the ratio of energy released to energy applied in such case of trigger action, we may ask the question, how does it come about that economic conversion factors, economic ratios-in-exchange of different forms of energy, display any regularity whatever?

We have been accustomed, in thermodynamics, to disregard mechanism. In dealing with energy conversions by trigger action we must make a complete change of attitude. Here everything depends on mechanism. If we find any degree of regularity in the conversion factors, this must be due to regularities in the mechanism, i.e., in the human organism, and its social aggregations; and we cannot hope to make any progress in our understanding of the physical principles involved except by taking due account of the mechanism.

In view of the extreme complexity of the systems in which economic phenomena have their course, it may appear hopeless to attempt analysis. But reflection shows that the complexity is in the details; the broad underlying features resolve themselves into comparatively simple lines.

Our vital interest in physical energy arises out of the following facts:

1. The body of a living organism is *not* in equilibrium with its surroundings. In other words, the living body (and also the dead body, for some time after death, as popularly conceived), in its natural environment, is a source of available energy.

2. Though not in equilibrium with its surroundings, the living body is in an approximately steady state; furthermore, it is one of the essential conditions for the continuance of life that this approximately steady state be maintained. Extreme departures therefrom result in permanent displacement from the steady state, and, ultimately, in the dissipation of the available energy presented in the substance of the organism.

3. In the course of events a certain amount of available energy, varying according to the nature of the organism, is unavoidably dissipated, per unit of time.

It follows that every organism, in order to maintain the steady state necessary for its survival, must be provided with devices for capturing available energy.

Furthermore, in the competition which takes place among organisms, the advantage must go to those whose energy-capturing devices are most effective in directing available energy into such channels as are favorable to the preservation of the species.

In man, as an organism of the animal type, the mechanism for capturing available energy comprises three elements:

1. Sense Organs, or Receptors, whose function is to establish a certain rather close correlation between the state of the environment and that of the individual. Their function, in fact, is to *depict* the external world in the organism, to apprise him of the state of his environment.

2. Organs of Operation, or Effectors, such as hands, feet, etc., by means of which the individual reacts physically upon his environment so as to modify it, or to modify his relation to it (as, for instance, by locomotion).

3. Organs and Faculties of Adjustment, or Adjustors, whereby the action of the Effectors is adjusted in accordance with the indications furnished by the sense organs (receptors), and with the needs of the organism.

Among these adjustor faculties one which figures prominently and plays an important rôle is what we may call the *sense of values*, that faculty which we exercise when we are confronted with two or more alternative courses of action, from which one must be selected. In such a situation we choose the course which appears to us, subjectively, the more *desirable*, and we do not ordinarily give any thought to the question as to what may be, objectively, the significance of "desirability," any more than we do ordinarily concern ourselves, in viewing a landscape, as to the particular wave-length, the objective characteristic, of the light which to our subjective judgment appears "green."

And yet, for the interest of the species, it is evidently far from indifferent what may be the objective characteristics of those things which to us appear, subjectively, desirable. Upon the proper adjustment of the sense of values, of the "tastes," to certain objective realities depends the welfare of the species. A nation of drunkards, for example, is not destined to figure among the winners in the struggle of evolution.

If, then, the physicist has been interested in discovering the objective significance of the indications of our sense organs, if he has thought it within his province to investigate the relation between color and wave-length, between musical pitch and frequency, then it is equally a problem for the physicist to enquire into the physical basis of economic value.

The ground has been prepared for this enquiry by the mathematical economists in their hedonistic calculus. A person having at his disposal a given sum of money, and being confronted with the necessity of choosing between different ways of spending it, will seek so to apportion his expenditures as to procure for himself a maximum of pleasure. So, for example, if the choice lies between two commodities C_1 and C_2 (which may, in particular, be certain forms of energy) he will purchase quantities q_1 and q_2, respectively, such that, if his total pleasure derived from the purchase is Ω, then

$$\frac{\partial\Omega}{\partial q_1} dq_1 + \frac{\partial\Omega}{\partial q_2} dq_2 = 0 \tag{1}$$

where dq_1, dq_2 are small, arbitrary increments in the quantities of the commodities C_1, C_2 purchased. If p_1, p_2 are the prices upon the open market, say, per unit quantity, of commodities C_1, C_2, respectively, then evidently the corresponding increment in expenditure is $p_1 dq_1 + p_2 dq_2$. But the sum of money to be spent is fixed. We must have, therefore,

$$p_1 dq_1 + p_2 dq_2 = 0 \tag{2}$$

It follows, then, from (1) and (2), that the individual will so apportion his purchases, that

$$\frac{\partial\Omega}{\partial q_1} \Big/ \frac{\partial\Omega}{\partial q_2} = \frac{p_1}{p_2}. \tag{3}$$

If the commodities C_1, C_2 are "necessities of life," whose influence upon the life of the species is to increase r, the proportional rate of increase of the species, then, evidently, the interests of the species demand that the purchases be so apportioned as to make r a maximum, and thus, by similar reasoning, to make

$$\frac{\partial r}{\partial q_1} \Big/ \frac{\partial r}{\partial q_2} = \frac{p_1}{p_2}. \tag{4}$$

If we regard the sense of values as a device for attaining, as nearly as may be, the adjustment (4), then we see that a perfect sense of values would make

$$\frac{\partial\Omega}{\partial q_1} \Big/ \frac{\partial\Omega}{\partial q_2} = \frac{\partial r}{\partial q_1} \Big/ \frac{\partial r}{\partial q_2}. \tag{5}$$

So that, in a community of organisms endowed with a sense of values thus perfectly adjusted, the marginal utilities $\frac{\partial\Omega}{\partial q}$ (and hence the prices on an open market) would be proportional to the partial derivatives $\frac{\partial r}{\partial q}$. In practise this proportionality will not be actually reached; but it represents the limiting state towards which a race must tend to evolve. For, a race having tastes radically at variance with the exigencies of race propagation (measured by the rate of increase r), could not long survive, as pointed out years ago by Herbert Spencer.[4]

It is this tendency to approximate (perhaps somewhat remotely) to the adjustment (5), which gives rise to fairly definite economic interconversion factors of different forms of energy, as purchased upon the market, and applied to specific uses. There is no need to invoke a specific "economic energy;" on the contrary, our analysis, in which the quantities $\frac{\partial r}{\partial q}$ a significant rôle, lends little support to Helm's view regarding the phy dimensions of economic value. Such value, or, to use more exact te "marginal utility," does not, in the analysis here presented, appear as intensity factor of an energy.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hy and Public Health, Johns Hopkins University, No. 40.

[2] Helm, G., *Die Lehre von der Energie*, Leipsic, 1887, pp. 72 et seq.

[3] Ostwald, W., *Energetische Grundlagen der Kulturwissenschaften*, Leipsic, 190 155. *Die Philosophie der Werte*, Leipsic, 1913, pp. 260, 314–317, 326, 328. A other writers who touch on the subject of the relation of economic value and pric energy are: Budde, *Energie und Recht*, Leipsic, 1902, p. 56; Winiarski, "Essay s Mécanique Sociale," *Revue Philosophique*, 1900, vol. 49, p. 113. See also J. David *Qu. J. Economics*, Aug., 1919, p. 717.

[4] And for others whose pains and pleasures he makes his own.

A SIMPLE GRAPHIC CONSTRUCTION FOR FARR'S RELATION BETWEEN BIRTH-RATE, DEATH-RATE, AND MEAN LENGTH OF LIFE

ALFRED J. LOTKA

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A SIMPLE GRAPHIC CONSTRUCTION FOR FARR'S RELATION BETWEEN BIRTH-RATE, DEATH-RATE, AND MEAN LENGTH OF LIFE*

By Alfred J. Lotka

In a previous article[†] the writer has discussed the rational basis Farr's rule[‡]

$$\frac{1}{3}\cdot\frac{1}{b}+\frac{2}{3}\cdot\frac{1}{d}=L$$

where

$b=$ birth-rate per head
$d=$ death-rate per head
$L=$ mean length of life.

Writing Farr's rule in the more general form

$$P\frac{1}{b}+Q\frac{1}{d}=L$$

or

$$p\frac{1}{b}+q\frac{1}{d}=1$$

$$(b-p)(d-q)=pq$$

it was shown in the paper cited how the values of P, Q (represented Farr's formula by the approximate numerical coefficients $\frac{1}{3}$ and $\frac{2}{3}$ could be determined exactly.

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[*] *Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public He Johns Hopkins University.* No. 46.

[†] *Quart. Pub. Am. Statis. Assn.*, Sept., 1918, p. 121.

[‡] Quoted by Newsholme, *Vital Statistics*, 1899, p. 301.

In the course of some work on malaria epidemiology now in progress and to be published elsewhere later, a somewhat similar relation between certain variables was encountered, leading to the following graphic construction.

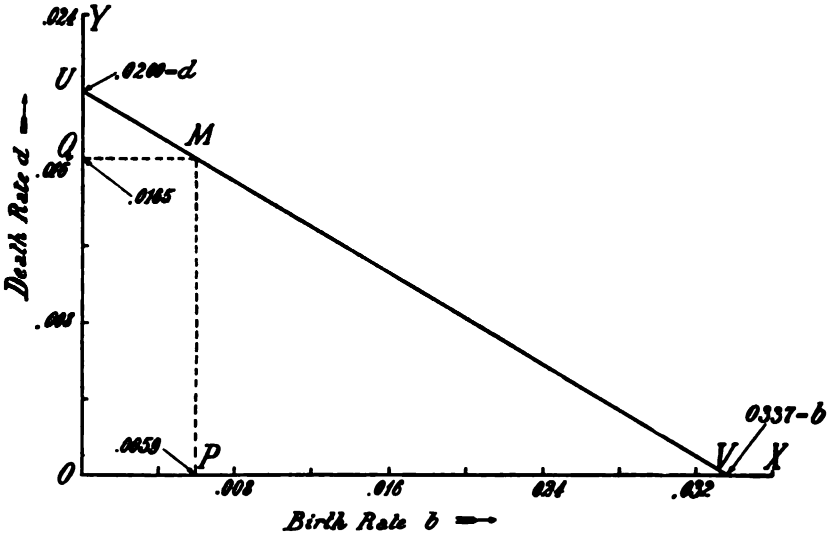

FIG. 1.

Along OX mark off a length $OP=p=\frac{P}{L}$ (8)

Along OY mark off a length $OQ=q=\frac{Q}{L}$ (9)

Complete the rectangle $QOPM$
Suppose we are now given

$$b=0.0337 \tag{10}$$

It is required to find d
Along OX mark off $OV=0.0337$ (11)
Join VM and produce it to meet OY in U
Read off at U on the scale of OY

$$d=0.0200. \tag{12}$$

The figures employed in this example are those for the female population of England and Wales 1871–80, a period during which (as the writer has previously shown) the observed figures for many characteristics of the population agree very closely with those computed for a "Natural Population Norm."* In the particular instance here chosen the numerical values were

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* *Jour. Washington Acad. Sci.*, vol. 3, May, 1913, pp. 241–89.

$$P = 0.2631 \quad (1$$

$$Q = 0.7369 \quad (1$$

$$L = 44.62 \quad ($$

$$\frac{P}{L} = 0.01651 \quad ($$

$$\frac{Q}{L} = 0.00590 \quad ($$

i. e.,

$$0.2631\,\frac{1}{b} + 0.7369\,\frac{1}{d} = 44.62. \quad ($$

Putting $b = 0.03373$ (observed) we find (

$$d = 0.02000 \text{ (calculated)} \quad ($$

as against

$$d = 0.020001 \text{ (observed)}. \quad ($$

I take this opportunity thus to supplement the data given for t male population in my previous paper. At the time it was writt the extraordinarily close agreement between calculated and obser figures for the female population had escaped my notice.

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THE GENERAL CONDITIONS OF VALIDITY OF THE PRINCIPLE OF LE CHATELIER.

BY ALFRED J. LOTKA.

THE GENERAL CONDITIONS OF VALIDITY OF THE PRINCIPLE OF LE CHATELIER.[1]

By Alfred J. Lotka.

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The derivation of the principle of Le Chatelier from the laws of thermodynamics is familiar.

We may approach a converse problem. What, in the broadest terms, are the conditions which a system must satisfy in order that the principle shall apply to it? The interest of this problem arises from the fact that we have reason to suspect these conditions may prove broader than the domain within which the laws of thermodynamics are conveniently applicable.[2] We may therefore expect that a satisfactory solution of the converse problem may enable us to make rigorous application of the principle to systems to which, from lack of sufficient data it may be impossible, or from other causes it may be inconvenient to apply thermodynamic methods.

Consider a system whose state is defined in terms of a variable x and a paramenter G. The system is one of that class, the history of which follows a law

$$\frac{dx}{dt} = f(x, G) \tag{1}$$

(For example, it may consist of a mixture of $(A_1 - 2x)$ mols H_2O vapor, $(A_2 + 2x)$ mols of hydrogen, and $(A_3 + x)$ mols of oxygen at 2000 deg. C. in a rigid enclosure of volume G; A_1, A_2, A_3 being constants, namely initial masses). It is understood that other parameters besides G may enter into the function f, but it is unnecessary to set them forth explicitly, since in the reflections which follow only changes in x and in one parameter G at a time will be considered, the other paramenters being constant.

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 37.

[2] See Ehrenfest, Zeitschr. für phys. Chem. 1911, vol. 77, pp. 227, 244; Wolchonsky, Jl. Russ. Phys. Chem. Soc., 1912, vol. 44, pp. 305, 310; Chwolson, Lehrbuch der Physik, 1909, vol. 3, p. 547; Bancroft, Jl. Am. Chem. Soc., 1911, p. 92; Fournier d'Albe, Contemporary Chemistry, 1911, p. 38; Löwy, Kosmos, 1911, p. 331; Le Dantec, La Stabilité de la Vie, 1910, p. 25; L. Fredericq, Arch. de Zool. Exp. et Gén., ser. 2, vol. 3, 1885, p. XXV; Spencer, First Principles, chapter 22, section 173, Burt's Edition, p. 433. For further historical and bibliographic notes see Duhem, Traité d'Énergétique, 1911, vol. 1, pp. 523, 524.

According to (1) a stationary state (which need not be a true equilibrium in the thermodynamic sense) is defined by

$$0 = \frac{dx}{dt} = f(x_1, G) \tag{2}$$

where x_1 denotes the equilibrium value of x.

If the parameter G is altered by a small increment δG, the corresponding increment δx_1 in the equilibrium value x_1 of x is, in view of (2), given by

$$\frac{\partial f}{\partial x}\,\delta x_1 + \frac{\partial f}{\partial G}\,\delta G = 0 \tag{3}$$

$$\frac{\delta x_1}{\delta G} = -\frac{\partial f}{\partial G}\Big/\frac{\partial f}{\partial x} \tag{4}$$

1. STABLE STATE.

If the stationary state defined by (2) is stable, we must have in the neighborhood of that state[3]

$$\frac{\partial f}{\partial x} < 0 \tag{5}$$

We can then distinguish two cases:

a.) $\frac{\partial f}{\partial G} > 0$. This means that the parameter G is one whose increase accelerates the transformation the progress of which is measured by x. In this case it follows immediately from (4) that $\frac{\partial x_1}{\delta G} > 0$. In other words, if the system is stable in the stationary state defined by (2), then increasing a parameter which accelerates the transformation will shift the position of the stationary state in the direction of increased transformation. From this alone, however, it does not necessarily follow that the new stationary state will actually become established. But, starting from the stationary state, at which $\frac{dx}{dt} = f = 0$, increase in G leads to a positive value of f. That is to say, a change actually takes place with a velocity directed towards the new stationary state, i.e. increased x.

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[3] Condition (5) states that the velocity $f = \frac{\partial f}{\partial x}\,\delta x$ is always opposite in sign to the (small) displacement δx from equilibrium. This is evidently necessary for stability of equilibrium.

b.) $\frac{\partial f}{\partial G} < 0$; i.e. increase in the paramenter G retards the transformation. Here it follows by similar reasoning that increase in G shifts the position of the stationary state towards diminished transformation. Furthermore, in this case the increment δG initiates a retrograde change, i.e. a change toward the new stationary state.

In both cases, (1.a) and (1.b), therefore, a change δG in the parameter G is followed by a transformation δx_1 towards the new stationary state, in the direction of the influence of the parameter G upon the velocity of transformation.

2. Unstable State.

Consider now the case in which $\frac{\partial f}{\partial x} > 0$. The stationary state defined by (2) is then unstable. A train of reasoning precisely analogous to that set forth above leads, in this case, to the conclusions:

(1) A change δG in the parameter G determines a shift of the stationary state in the direction opposed to the influence of the parameter G upon the transformation.

(2) The system, disturbed from existing stationary state by a change δG, moves, not towards, but away from the new stationary position.

Application to Influence of Initial Masses. Consider a transformation

$$S_1 + S_2 + \ldots + S_r \rightleftarrows S'_1 + S'_2 + \ldots + S'_s \tag{6}$$

Let $\xi_1, \xi_2, \ldots \xi_r$ be the masses (expressed in mols) at time t of the components $S_1, S_2 \ldots S_r$; similarly let $\xi'_1, \xi'_2 \ldots \xi'_s$ be the masses of $S'_1, S'_2 \ldots S'_s$

Let x measure the progress of the transformation from left to right, and let $p_i\, x$ be the amount (in mols) of S_i transformed from time $t = 0$ to time $t = t$.

Let A_i be the initial value of the mass (in mols) of some component

S_i which disappears in the transformation when x increases, and let A'_j be the initial value of some component S'_j which appears in the same transformation.

We have, according to (4),

$$\frac{\delta x_1}{\delta A_i} = -\frac{\partial f}{\partial A_i}\Big/\frac{\partial f}{\partial x} \tag{7}$$

If we are dealing with a system of constant mass, we have an equation of constraint

$$m_1\xi_1 + m_2\xi_2 + \ldots = m_1A_1 + m_2A_2 + \ldots \tag{8}$$

where $m_1, m_2, \ldots$ are the molecular weights of the substances $S_1, S_2, \ldots$ From (8) we find by differentiation

$$\frac{\partial \xi_i}{\partial A_i} = 1 \tag{9}$$

so that we may write, instead of (7),

$$\frac{\delta x_1}{\delta A_i} = -\frac{\partial f}{\partial \xi_i}\Big/\frac{\partial f}{\partial x} \tag{10}$$

From (10) it is seen that $\frac{\delta x_1}{\delta A_i}$ and $\frac{\partial f}{\partial \xi_i}$ are always of the same sign provided $\frac{\partial f}{\partial x}$ is negative, i.e., provided that the system is stable in the equilibrium defined by $f = 0$. That is to say, if the system is stable, and if adding a quantity of a component disappearing in the transformation increases the velocity of the transformation (at the previous equilibrium), then such addition will shift the equilibrium in the direction of increased transformation. In this case, then, the principle of Le Chatelier holds good.

On the contrary, by similar reasoning, it is found that if the addition of a quantity of a particular substance disappearing in the transformation retards the transformation, the principle does not hold as regards that substance.

Again, by similar reasoning, it is found that the principle holds or does not hold, according as the addition of a substance S'_j appearing in the transformation retards or hastens the transformation. We may therefore summarize the facts as follows:

1. Given that the system, at equilibrium, is stable with regard to changes in x, and that there is a relation of the type (8), an "equation of constraint" connecting the masses ξ and their initial values A, then the condition which must be satisfied in order that the Le Chatelier Principle may hold with regard to the effect of a change in the initial mass of some *one* component, is that the addition of such component shall accelerate or retard the transformation (at equilibrium), according as such component disappears or appears in such transformation.

2. Given that the conditions for the validity of the Le Chatelier Principle stated under (1) are satisfied for each and every component, then it is easily shown that the system is necessarily stable with regard to changes in x, so that the condition of such stability with regard to changes in x is automatically satisfied and does not need to be expressly stated. For, if $\frac{\partial f}{\partial A_i} > 0$ for every component which disappears in the transformation, and if $\frac{\partial f}{\partial A'_j} < 0$ for every component which appears, then, in view of (9), the same is true of $\frac{\partial f}{\partial \xi_i}$ and $\frac{\partial f}{\partial \xi'_j}$.

But

$$d\xi_i = - p_i \, dx \tag{11}$$

$$d\xi'_j = + p'_j dx \tag{12}$$

where p_i, p'_j are positive numbers, and

$$\frac{df}{dx} = \Sigma \frac{\partial f}{\partial \xi_i} \frac{d\xi_i}{dx} + \Sigma \frac{\partial f}{\partial \xi'_j} \frac{\partial \xi'_j}{dx} \tag{13}$$

$$= - \Sigma \frac{\partial f}{\partial \xi_i} p_i + \Sigma \frac{\partial f}{\partial \xi'_j} p'_j \tag{14}$$

which is necessarily a negative quantity if

$$\frac{\partial f}{\partial \xi_i} > 0 , \quad \frac{\partial f}{\partial \xi'_j} < 0 \tag{15}$$

3. It should be noted that the argument by which our conclusions have been drawn depends on the existence of equations of constraint, relations such as (8), connecting the ξ's and the A's. In the absence

of such constraints we are in no wise assured that the principle holds.[4] This must be clearly borne in mind in seeking to apply the Le Chatelier principle, for example, to biological systems. Thus, for instance, the malaria equilibrium under the conditions contemplated by Sir Ronald Ross,[5] is independent of the initial amount of malaria in the system (provided only this is not zero). This state of affairs arises out of the fact that there is no equation of constraint of type (8), in this case, connecting the initial amount of malaria with its status at any subsequent epoch.

Case of more than one variable. A somewhat more complicated case arises if the system under consideration is susceptible of several concurrent transformations, so that its state at any instant requires for its definition not one variable x, but a number of such variables.

It will suffice if we consider here the case for two variables x, y, as, for example, the case of a pair of consecutive reversible reactions

$$A \rightleftarrows B \rightleftarrows C \tag{16}$$

In this case we have

$$\frac{dx}{dt} = f_1(x, y, G) \tag{18}$$

$$\frac{dy}{dt} = f_2(x, y, G) \tag{19}$$

and equilibrium is defined by

$$f_1 = f_2 = 0 \tag{20}$$

Differentiating, in a manner analogous to that followed in the case of a single variable x, we have

$$\frac{\partial f_1}{\partial x}\,\delta x_1 + \frac{\partial f_1}{\partial y}\,\delta y_1 + \frac{\partial f_1}{\partial G}\,\delta G = 0 \tag{21}$$

$$\frac{\partial f_2}{\partial x}\,\delta x_1 + \frac{\partial f_2}{\partial y}\,\delta y_1 + \frac{\partial f_2}{\partial G}\,\delta G = 0 \tag{22}$$

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[4] For there is then no necessary relation between ξ and A, so that the derivative $\frac{\partial f}{\partial A}$ is no longer equal to $\frac{\partial f}{\partial \xi}$, but is indeterminate or meaningless.

[5] "The Prevention of Malaria," Second English Edition, John Murray, London, 1911, p. 679; Lotka, Nature, Feb. 1912, p. 497.

a system of linear equations, which we solve for $\frac{\delta x_1}{\delta G}, \frac{\delta y_1}{\delta G}$ and obtain

$$\frac{\delta x_1}{\delta G} = - \frac{\begin{vmatrix} \frac{\partial f_1}{\partial G} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial G} & \frac{\partial f_2}{\partial y} \end{vmatrix}}{\begin{vmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y} \end{vmatrix}} \tag{23}$$

and a similar expression for $\frac{\delta y_1}{\delta G}$.

Condition of Stability. A general solution of (18), (19) can be written [6] in the form of exponential series

$$x = P_0 + P_1 e^{\lambda_1 t} + P_2 e^{\lambda_2 t} + P_{11} e^{2\lambda_1 t} + \dots \tag{24}$$

$$y = Q_0 + Q_1 e^{\lambda_1 t} + Q_2 e^{\lambda_2 t} + Q_{11} e^{2\lambda_1 t} + \dots \tag{25}$$

where λ_1 λ_2 are the roots of

$$\Delta(\lambda) = \begin{vmatrix} \left(\frac{\partial f_1}{\partial x} - \lambda\right) & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \left(\frac{\partial f_2}{\partial y} - \lambda\right) \end{vmatrix} = 0 \tag{26}$$

The condition for stability [7] of the equilibrium is that the real parts of all the roots λ are negative. This in turn demands that the absolute term $\Delta(0)$ be positive. But this absolute term is, evidently,

$$\Delta(0) = \begin{vmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y} \end{vmatrix} \tag{27}$$

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[6] A. J. Lotka, Proc. Am. Ac., 1920, p. 139.

[7] Idem, loc. cit., p. 144; Hurwitz, Math. Ann., 1875, vol. 46, p. 521; Blondel, Jl. de Physique, 1919, pp. 117, 153.

so that we must have, for stability,

$$\begin{vmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y} \end{vmatrix} > 0 \tag{28}$$

In consequence, given stability of equilibrium, the sign of $\frac{\partial x_1}{\partial G}$ will be the same as that of the numerator in (23), i.e., that of the expression

$$\frac{\partial f_1}{\partial G}\frac{\partial f_2}{\partial y} - \frac{\partial f_2}{\partial G}\frac{\partial f_1}{\partial y} = D \tag{29}$$

Example. Consecutive Reactions. By the way of example we may apply these results to the case of a pair of consecutive reversible reactions.

$$S_1 + S_2 + \ldots + S_r \rightleftarrows S'_1 + S'_2 + \ldots + S'_s \rightleftarrows S''_1 + S''_2 + \ldots + S''_t \tag{30}$$

Let x denote the progress of the first reaction from left to right (so that, for example, a quantity, $p_i x$ of the substance S_i has been transformed at time t); and let y similarly denote the progress of the second reaction, from left to right.

Let us consider the effect upon x_1, the equilibrium value of x, of an increment $\delta A''_k$ in the initial amount of substance S''_k appearing as product of the second reaction.

We have, according to (23)

$$\frac{\delta x_1}{\delta A''_k} = - \frac{\begin{vmatrix} \frac{\partial f_1}{\partial A''_k} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial A''_k} & \frac{\partial f_2}{\partial y} \end{vmatrix}}{\begin{vmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y} \end{vmatrix}} \tag{31}$$

We shall assume stability, so that the denominator is positive.

In the numerator, evidently [8]

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[8] If we exclude any possible catalytic influence.

$$\frac{\partial f_1}{\partial A''_k} = 0 \tag{32}$$

so that this numerator reduces to

$$\frac{\partial f_2}{\partial A''_k} \cdot \frac{\partial f_1}{\partial y} \tag{33}$$

Stability demands

$$\frac{\partial f_1}{\partial y} > 0 \tag{34}$$

On the other hand the principle of Le Chatelier would make

$$\frac{\delta x_1}{\delta A''_k} < 0 \tag{35}$$

This, by (31), in view of (32), (33), (34), will be true or not according as

$$\frac{\partial f_2}{\partial A''_k} \substack{<\\>} 0 \tag{36}$$

Hence the principle of Le Chatelier holds good or not, as applied to the effect of A''_k upon x_1, according as

$$\frac{\partial f_2}{\partial A''_k} \substack{<\\>} 0 \tag{37}$$

From this point on the discussion would follow essentially similar lines as in the case of a single dependent variable; it is therefore unnecessary to carry this out in further detail.

Influence of External Factors. We have hitherto tacitly assumed that (1), or (18), (19) are the only conditions for equilibrium, or, that, if there are any other conditions to be satisfied, these are in some way automatically taken care of.

In point of fact, in general, in addition to a condition of the form

$$\frac{dx}{dt} = f(x, G) \tag{1}$$

there will be further conditions of the form

$$H = H_e \tag{38}$$

where H is a parameter entering into the function f, while H_e is a parameter defining certain "external conditions." For example, H may be the pressure exerted by a gaseous mixture against an enclosure, and H_e may be the external pressure applied to a movable piston forming part of that enclosure. Here it is not enough, for complete equilibrium, that (1) be satisfied, but (38) also must hold.

Furthermore, the conditions (1) and (38) define equilibrium, but are insufficient to determine its stability, since they give us no information regarding the behavior of the system when $H \neq H_e$, i.e. when not in equilibrium with the external parameter H_e.

In order to settle this point we must have some further data. We are here interested in systems in which such additional data are furnished in the following manner:

In the case of these systems it is found that, in relation to the parameter G a certain parameter H having certain peculiar properties, can be defined by a relation.

$$\varphi\,(\xi_1, \xi_2, \ldots\ldots G, H) = \text{constant} \qquad (39)$$

or its equivalent

$$\Psi\,(x, A_1, A_2, \ldots G, H) = \text{constant} \qquad (40)$$

The peculiar property of G referred to above is as follows

$$\frac{dG}{dt} \gtreqless 0 \text{ according as } H - H_e \gtreqless 0 \qquad (41)$$

It will perhaps be well, before proceeding any farther, to illustrate this by a concrete example. Consider the system

$$2\,H_20 \rightleftarrows 2\,H_2 + 0_2 \qquad (42)$$

If ξ_1 is the mass of H_20 expressed in mols, ξ'_1 the mass of H_2 and ξ'_2 the mass of 0_2 similarly expressed; if V is the volume (parameter G) and if P is the pressure (parameter H) exerted upon the enclosure, then the equation (39) here takes the form

$$PV = (\xi_1 + \xi'_1 + \xi'_2)\,R\theta \qquad (43)$$

where θ is the absolute temperature and R the general gas constant. Or, if A_1, A'_1, A'_2 are the initial masses of H_20, H_2 and 0_2 respectively, (expressed in mols), and x measures the progress of the reaction, as, for example, by the number of 0_2 mols formed, then evidently

$$\xi_1 = A_1 - 2x \qquad (44)$$

$$\xi'_1 = A'_1 + 2x \qquad (45)$$

$$\xi'_2 = A'_2 + x \qquad (46)$$

so that (40) takes the form

$$\begin{aligned} PV &= \{(A_1 - 2x) + (A'_1 + 2x) + (A'_2 + x) \quad R\theta \\ &= \{(x + A_1 + A'_1 + A'_2)\ R\theta \end{aligned} \tag{47}$$

In this case it is quite evident that the parameters P, V (corresponding to H, G of the general case) have the property defined by (41), which here appears as the characteristic property of the intensity factor and the capacity factor of an energy.

But for our present purposes we are not concerned with the question whether or not the parameters G, H defined for a given system are or are not factors of an energy. We must be prepared to deal with cases where this is either uncertain or actually known not to be true. All we need to know, for our purpose, is that the parameters G, H have the property defined by (41). An example may serve to illustrate the fact that this property may be shared by physical quantities not obviously related to energy.

Among the parameters on which the rate of increase of a human population depends is the area a occupied by them, since this determines the population density, which in turn influences the death rate in well-known manner,[9] and, presumably, in some degree the birth rate also.

Now there is an obvious relation between population density and ground rent. Regulation is effected about as follows: There is a certain demand for space, a desire for expansion, which may be measured by the rent H per unit area that the individual is willing to pay. On the other hand there is a certain market price H_e which must be paid to obtain accommodation. Now if $H > H_e$, i.e. if, on an average, an individual is willing to pay more than the market price, the population will spread over a greater area by renting more ground. If, on the other hand $H < H_e$ the individual is not willing to pay the market price, he will retrench, he will move from a six room apartment to a five room apartment say, and the area occupied by the population will contract. The parameter H_e functions, in fact, much like a "surface pressure," tending to compress the population into a smaller area. The most striking exhibition of this "surface-pressure" is seen in a great metropolis such as New York, where the population, a naturally two-dimensional structure spread like a film over the earth's surface, has been thrown into great creases towering 700 feet and more, 50 layers deep, above the street level.

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[9] See, for example, Newsholme, Vital Statistics, 1899, p. 154.

It will be seen that in this case the internal parameter H and the corresponding external parameter H_e so determine changes in the area a that

$$\frac{da}{dt} \gtreqless 0 \text{ according as } H - H_e \gtreqless 0 \qquad (48)$$

that is to say, the parameters H, a and H_e are related to each other and determine the course of events in a manner analogous to the intensity factor, the capacity factor of an energy, and the "applied force." But it is quite unnecessary to suppose that H and a actually are such factors of an energy in the example cited (population-spread); on the contrary, the writer is opposed to this view, which he has taken occasion elsewhere to discuss.[10] For our purposes it is quite immaterial whether P and a are factors of an energy. All we need know is that they enter into the condition (41) as there set forth.

Condition for Stability toward External Factor. Consider a system for which the condition for equilibrium with the environment is given by

$$\left.\begin{array}{l} H = H_e \\ \varphi(G, H) = \text{constant} \\ \dfrac{dG}{dt} \gtreqless 0 \text{ according as } H - H_e \gtreqless 0 \end{array}\right\} \qquad (49)$$

Let

$$\varphi(G, H) = \text{const., i.e.} \quad H = \chi(G) \qquad (50)$$

be plotted as ordinates in a rectangular system in which G is plotted as abscissae. Then it is easily shown that the condition for stability of equilibrium is that the curve $H = \chi(G)$ must slope downwards from left to right.

For, suppose it sloped upwards. Let the system be in equilibrium at a point A_1 (Fig. 1), where

$$\left.\begin{array}{l} H = H_1 = H_e \\ G = G_1 \end{array}\right\} \qquad (51)$$

Suppose the system is in any way displaced to the point A_2 where

$$H_2 > H_1 \qquad (52)$$

$$> H_e \qquad (53)$$

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[10] "Economic Conversion Factors of Energy," to appear in a forthcoming issue of Proc. Nat. Ac.

Then

$$\frac{dG}{dt} > 0 \qquad (54)$$

Hence the point moves along the curve in the direction $A_1 A_2$, i.e. still farther away from equilibrium.

On the other hand, the same reasoning applied to a curve sloping downward from left to right shows that the system after displacement returns to its equilibrium position.

So, for example, the curves representing the relation between pressure and volume of a gas necessarily slope downward from left to right; the same is true of the demand and supply curves of economics. If it were true, as sometimes stated, that the more a man has, the more he wants, economic equilibrium would be an unstable condition.

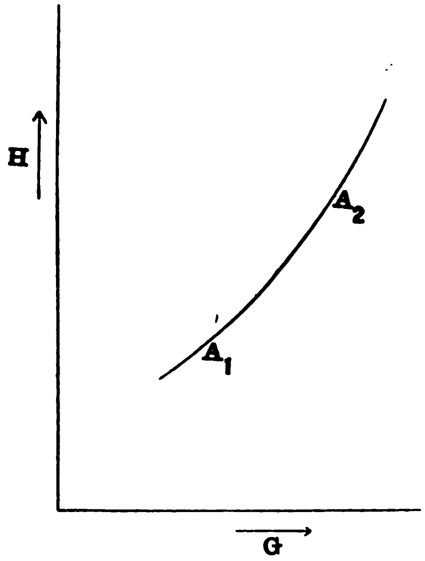

FIGURE 1.

External Stability and the Principle of Le Chatelier. Consider now a system which obeys the condition

$$\left.\begin{array}{l}\varphi(x, G, H) = 0 \\ \dfrac{dG}{dt} \gtreqless 0 \text{ according as } H - H_e \gtreqless 0\end{array}\right\} \qquad \begin{array}{r}(55)\\(56)\end{array}$$

Let the system be stable towards H_e both when x is held constant and also when x is at the equilibrium value x_1 defined by

$$\frac{dx}{dt} = f(x_1, G) = 0 \tag{57}$$

This means that all the curves "of constant composition"

$$\left.\begin{aligned} \varphi(x, G, H) &= 0 \\ x &= \text{constant} \end{aligned}\right\} \tag{58}$$

and also the curve "of equilibrium composition"

$$\varphi(x_1, G, H) = 0 \tag{59}$$

slope from left to right downwards.

Now consider two neighboring curves of type (58) (curves of constant composition), which we will suppose solved for H and write

$$H_a = \Psi_a(G, x_a) \tag{60}$$

$$H_b = \Psi_b(G, x_b) \tag{61}$$

Suppose we start with the system in the state represented by the point Q, in internal equilibrium and also in equilibrium with an external parameter H_e (see Fig. 2).

Let x be changed at constant G, so as to increase H according to (55) until $x = x_b$, so that the representative point strikes the second curve of constant composition at R.

Since at the start of this operation

$$H = H_a = H_e \tag{62}$$

and at the end

$$\left.\begin{aligned} H &= H_b \\ &> H_e \end{aligned}\right\} \tag{63}$$

therefore the system is not in equilibrium with the external pressure H_e in the state represented by the point R, but equilibrium (for $x = x_b$) occurs at some other point T which must lie to the right of R along the curve of constant composition RT, since, whenever

$$H > H_e$$

G increases, in accordance with (56).

Furthermore, drawing a horizontal QS, T must lie below S, since the line of equilibrium composition QT must slope from right to left downwards.

It is clear therefore that lines of constant composition are steeper than lines of equilibrium composition.

It follows at once that if G be increased while the system is kept in equilibrium, so that the representative point travels along QT, then the change in x from x_a to x_b is that which at constant G increases H, or at constant H increases G.

But this is the principle of Le Chatelier. This principle therefore holds whenever the conditions (55) (56) are satisfied, and the system is stable towards H_e both when x = constant and when $x = x_1$ (i.e. when x has its equilibrium value).

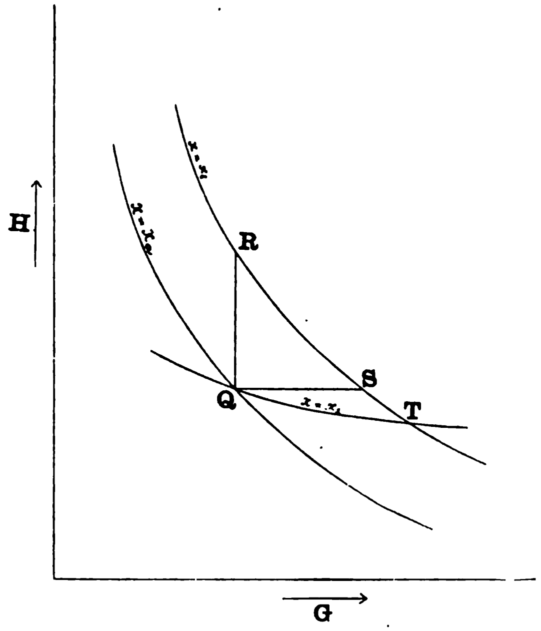

FIGURE 2.

Similarly, it can be shown that if the conditions (55), (56) are replaced by

$$\left.\begin{aligned}&\varphi(x, G, H) = 0\\&\frac{dG}{dt} \begin{smallmatrix}<\\=\\>\end{smallmatrix} 0 \text{ according as } H - H_e \begin{smallmatrix}>\\=\\<\end{smallmatrix} 0\end{aligned}\right\}$$

while at the same time the system is stable towards H both when x = constant and also when $x = x_1$, then the Le Chatelier principle holds. In this case the curves $\varphi\ (x,\ G,\ H) = 0$ (x constant)

and $$\Psi\ (x_1, G, H) = 0$$

both slope from left to right upwards, and the curves of constant composition are again steeper than the equilibrium curves. An example of this type is that in which H is temperature and G is heat absorbed by the system (when $H < H_e$, G increases).

Finally, be it remarked that the results here deduced depend solely on *kinetic stability*, i.e. on the fact that the system when displaced from equilibrium has a velocity (rate of change of displacement) towards that equilibrium. The conclusions reached are therefore wholly independent of energetic (thermodynamic) consideration, since no reference whatever has been made to forces or energies or in any way whatsoever to the physical dimensions of the parameters involved.

This completes the present enquiry into the conditions of validity of the principle of Le Chatelier. It remains now only to point out the place which this communication occupies in the general plan of the series of investigations of which it forms part. This series of investigations has for its object the study of material systems evolving in accordance with a system of differential equations

$$\frac{dX_i}{dt} = F_i(X_1, X_2, \ldots X_n;\ A;\ P;\ Q) \tag{65}$$

in which the symbols X denote the masses of certain components S of the system, the symbol A has been written to denote collectively the initial values of the masses of certain components, the P's are parameters defining the state of the system (extension-in-space, topography, climatic conditions, etc.); and the Q's are parameters defining the character of the components S.

In a previous communication the kinetics of such a system were studied for the case in which the A's, P's and Q's are constant. This left open for discussion the effect of changes in these parameters. One phase of this subject has been dealt with by the writer elsewhere,[11] namely the effect of slow changes in these parameters. The present communication now extends the field of enquiry to the effect of changes of any kind in the parameters A or P, with this restriction, it is true, that we have, following Le Chatelier, interested ourselves solely in the ultimate effect upon equilibrium, leaving entirely out of consideration the path by which such equilibrium is reached. In many cases such partial information is of value, the equilibrium being the matter of chief interest, the path of the change of lesser practical importance. Herein lies the utility of such general principles as that of Le Chatelier, of Maupertuis, Lenz and others whose names have become linked with one or other form of reciprocal relations between the parameters defining a state of equilibrium. Since the application of such principles to biological systems has been essayed by various authors, without, however, any rigorous foundation upon which to build, it has appeared to the writer essential, for further progress in this field, to make a critical examination of the basis underlying these principles.

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[11] "Note on Moving Equilibria"; to appear in a forthcoming issue of the Proc. Nat. Ac.

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THE INFLUENCE OF AGE ON FERTILITY IN SWINE[1]

BY TAGE ELLINGER
COPENHAGEN, DENMARK

Communicated by R. Pearl, March 10, 1921

In the breeding of domestic animals the problem of fertility is of prime portance. The neglect of this essential factor has again and again sed breeders disappointments and often heavy economic losses. Thus is desirable to study this chapter of the physiology of reproduction roughly and to examine the factors, internal and external, that affect e ability to produce offspring.

During an investigation on these subjects, in this laboratory the re-lts of which are soon to be published, the writer has been confronted with the question of the influence of age of the sow on the size of her litters. Although a number of records on this and similar subjects have been published from time to time, the writer is of the opinion that the special qualities of his material, and the mathematical treatment of it that it is possible to employ, justify this further publication.

In going over the literature on the influence of age on fertility in mammals, a number of records bearing on the question can be collected. Sarah V. Jones and James E. Rouse[10] have in a recent paper give an extensive summary of the literature, to which paper I only need to refer for further information.

The records published are of two different kinds. First: results of experiments chiefly with rats (King[11]), rabbits (Hammond[8]), and guinea-pigs (Minot[14]). The experiments on swine by Mumford[15] (1917) are of further interest in this connection.

The second group consists of statistical investigations generally using as material the numerous records given in the herd books of the different breeds of domestic animals.

Carlyle and Mc Connell,[6] Pearl,[16] Bell,[3,4] Humphrey and Kleinheinz,[9] Jones and Rouse[10] give records showing that the percentage of multiple births in sheep increases with age up to five years and then again declines. Pearl[16] and Jones and Rouse[10] give similar data for cattle showing a steady increase in the percentage of twin births with age.

Swine have often been subject to investigations of this kind. One of two sources of error have always been present in these. 1. The number of individuals involved have been so limited as to make dependable results unobtainable, or 2. The method of computing has been so rough as to give room for the effect of selection. The usual way of approaching the problem has been to take from herd book records of all available litters and to refer them to the age of their mothers at their birth. In this way it is possible to get a great number of litters from young sows but rather few from older ones as the less desirable animals are disposed of early. The older sows represent only a selected group of the best of those recorded at an early age, and the average size of litters at different ages of the sows are for that reason not directly comparable. Owing to these difficulties the results are very contradictory and uncertain. Rommel[18] found a steady increase in size of litter from one to five years. Frölich and Georgs[7] report a maximum in the second litter and then a decrease in size of the the litters. Machens[12] claims a maximum at the fourth litter. Carmichael and Rice[5] give a table showing an increase in fertility to the third year followed by a decrease.

2. The material used in this investigation consists of 134 sows of native Danish breed[2] which *all* have records from all of their first ten litters. The

records are taken from the Danish herd book Vols. V and VII. All these sows have been kept in state-supervised *breeding centres* under uniform conditions and care. They have all farrowed the first time at about one year of age and from then on been bred regularly producing ca. 2$^1/_4$ litter per year, practically without regard to season. The successive litters will for that reason be highly correlated with age, but it seems most convenient to use the litter-number as unit. The average of all litters, being 11.5 pigs, is far above the average shown by swine of the American type, and although these 134 sows undoubtedly are selected group, the great fertility is one of the outstanding and valuable qualities of the breed.

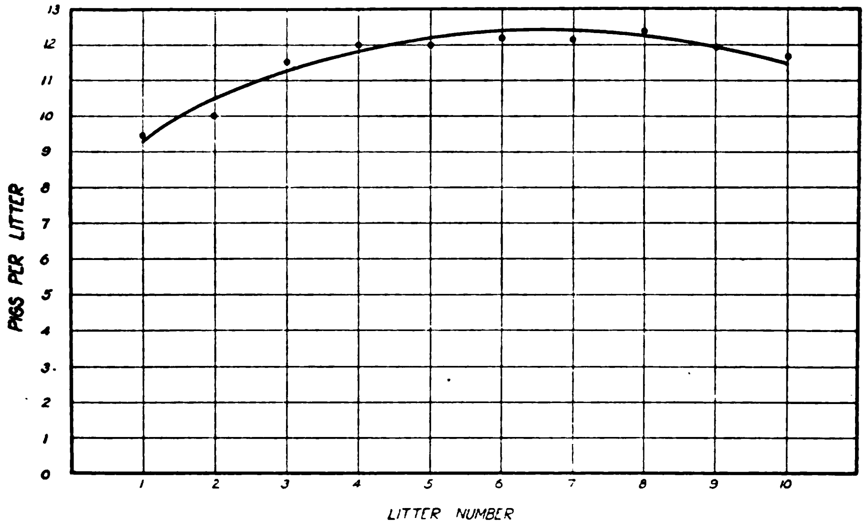

FIG 1.

The dots indicate the actual found averages for litter-size in the first ten litters. The curve is fitted to these valves.

In the second column of table 1 the average sizes of the successive ten litters are given.

TABLE I

LITTER NO.	AVERAGE SIZE OF LITTER	THEORETICAL SIZE OF LITTER
1	9.45	9.25
2	10.01	10.42
3	11.50	11.24
4	12.01	11.81
5	11.99	12.19
6	12.16	12.38
7	12.13	12.39
8	12.34	12.24
9	11.90	11.92
10	11.66	11.43
Average	11.52	11.53

Plotting the series of average values over a base indicating the successive generations (fig. 1) we see that they show an increase in fertility with age to a certain point followed by a decline. An empirical curve following these plotted points shows a type familiar to us from other biological problems. It is in fact a logarithmic curve. Pearl[17] has shown that the changes in milk flow with age in dairy cattle follow such a logarithmic curve and he has determined its equation.

The writer has now proceeded to form the equation relating fertility to to age in the material at hand, and has in the calculations used the method of moments (Miner[18]).

The general form of the equation is

$$y=a+bx+cx^2+d\log x$$

where y denotes the size of litter and x the ordinal litter number.

In this special case the equation has been calculated to be the following

$$y=8.414+0.915x-0.078x^2+1.627\log x.$$

By solving the equation for the values of x ranging from 1 to 10 the theoretical average size of the successive litters has been determined. These values are given in the third column of table 1 and they are used in plotting the curve in figure 1. This curve shows a satisfactory agreement with the empirical figures plotted out.

By putting $dy/dx = 0$ and solving for x the litter of maximum size is found to be 6.56th in order.

Summary.—1. Records from 134 sows of native Danish breed all having ten litters to their credit show an increase in fertility up to the 6.56 point in litter order followed by a decrease.

2. The curve relating fertility to age is logarithmic, its equation is calculated, and the curve fitted.

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 35.

[2] The native Danish breed is a white, fertile and hardy hog of bacon type, producing sometimes crossed with Yorkshire boars—the great proportion of the choice bacon exported from Denmark to England.

[3] Bell, A. G. 1904. The multi-nippled sheep of Beinn Bhreagh. *Science, N. S.*, 19.

[4] Bell, A. G. 1912. Sheep breeding experiments on Beinn Bhreagh. *Ibid.*, 34.

[5] Carmichael, W. J. and John B. Rice. 1920. Variation in farrow: With special reference to birth weight of pigs. *Univ. Illinois, Agric. Exp. Sta., Bull.* 226.

[6] Carlyle, W. L. and T. F. Mc Connell. 1902. Some observations on sheep breeding from the Experiment Stations flock records. *Univ. Wisconsin, Agric. Exp. Sta., Bull.* 95.

[7] Frölich und Georgs. 1911. Fruchtbarkeit und Geschlechtverhältnis beim weissen Edelschwein. *Jahrbuch für wissenschaftliche und praktische Tierzucht,* 6.

[8] Hammond, John. 1914. On some factors controlling fertility in domestic animals. *J. Agric. Sci.*, 6.

[9] Humphrey, G. C. and F. Kleinheinz. 1907. Observations on sheep breeding from records of the university flock. 24th *Ann. Rep. Wisconsin Agric. Exp. Sta.*

[10] Jones, S. N. H. and J. E. Rouse. 1920. The relation of dam to observed fecundity in domestic animals. I. Multiple birth in cattle and sheep. *J. Dairy Sci.*, 3.

[11] King, H. D. 1916. The relation of age to fertility in rat. *Anat. Rec.*, **11**.

[12] Machens, A. 1915. Fruchtbarkeit und Geschlechtsverhältnisse beim vereddelten Landschwein. *Berliner Tierärtsliche Wochenschrift*, **31**.

[13] Miner, J. R. 1915. Fitting logarithmic curves by the method of moments. *J. Agric. Res.*, **3**.

[14] Minot, C. S. 1891. Senescence and rejuvenescence. First paper: On the weight of guinea pigs. *J. Physiol.*, **12**.

[15] Mumford, F. B. 1917. *The Breeding of Animals.*

[16] Pearl, R. 1913. Note regarding the relation of age to fecundity. *Science, N. S.*, **37**.

[17] Pearl, R. 1914. On the law relating milk flow to age in dairy cattle. *Proc. Soc. Exp. Biol. Med.*, **12**.

[18] Rommel, G. M. 1907. Inheritance of litter size in Poland China sows. *American Breeders Report* **3**.

NOTE RELATIVE A L'ACTION PHOTODYNAMIQUE DE L'HÉMATOPORPHYRINE SUR LE FIBRINOGÈNE,

(*School of Hygiene and Public Health, Johns Hopkins University, Baltimore, U. S. A.*)

PAR

W.-H. HOWELL

Les effets photodynamiques de l'hématoporphyrine tels qu'ils furent décrits par HAUSMANN (¹) sur les infusoires, les érythrocytes et les souris ont été confirmés par divers observateurs, mais jusqu'à présent une explication satisfaisante de cette réaction nous manque. L'action de l'hématoporphyrine sur les souris, action sensibilisante vis-à-vis de la lumière, est particulièrement difficile à comprendre dans l'état où nous la montre une synthèse des principaux faits observés. Une dose d'hématoporphyrine aussi faible que 2 mgr. injectée sous la peau d'une souris blanche, se montre fatale en peu d'heures si l'animal est exposé à une vive lumière, tandis que très souvent cette dose n'est pas nuisible si la souris est maintenue dans l'obscurité. Bien plus : un animal traité par l'hématoporphyrine, mais conservé à l'obscurité, peut montrer l'effet photodynamique s'il est exposé à la lumière à n'importe quel moment de la première quinzaine ou plus longtemps après l'injection, bien que toutes les traces décelables d'hématoporphyrine aient disparu de l'organisme.

Les symptômes que montrent les animaux ont été décrits en détail par HAUSMANN. Dans mes propres observations, limitées à ce que HAUSMANN appelle les cas aigus, l'animal montre d'abord une congestion des oreilles et des pattes ; cette congestion disparaît dans la suite. Puis surviennent un œdème très marqué autour du nez et de la bouche et une irritation cutanée de la région céphalique, démontrée par les mouvements de grattement de l'animal. Ces symptômes disparaissent plus tard en tout ou en partie, mais on voit persister la photophobie qui se manifeste par l'occlusion des yeux et par une préférence pour les zones ombragées de la cage occupée par les animaux. Parfois l'animal tomba dans un état léthargique, interrompu de temps à autre par des mouvements spasmodiques, et la respiration se ralentit jusqu'à ce que la mort survienne. Pendant cette ultime période, il doit exister un degré marqué d'insensibilité, car on observa que, dans cet état, les souris ne réagirent par aucun trouble sensoriel évident lorsque des animaux de contrôle placés dans la même cage leur endommageaient les oreilles.

Dans mes expériences, chez quelques animaux injectés au moyen d'une préparation impure non cristallisée d'hématoporphyrine, j'ai observé au niveau des yeux l'apparition d'une cataracte. L'examen *post mortem* montra une opacité limitée à la couche toute superficielle du cristallin, dans la région équatoriale qui est en contact avec les procès ciliaires. Si nous songeons que dans ces expériences la lumière ne peut pénétrer d'une façon appréciable au delà du plexus sous-cutané des vaisseaux sanguins, nous devons admettre, pour expliquer les effets mortels, que des substances toxiques se forment probablement dans la peau elle-même (ainsi que Jesionek (2) le pense), sous l'influence combinée de l'hématoporphyrine et de la lumière, et que ces substances exercent ensuite leur effet sur le système nerveux central, soit par la voie sanguine, soit encore par un phénomène réflexe.

Vu que les faits que nous connaissons jusqu'à présent sont insuffisants pour nous permettre une explication de cette curieuse réaction, il est utile d'étendre les expériences et de collectionner toutes les influences susceptibles d'agir sur le phénomène.

Le but principal de la présente note est d'attirer l'attention sur quelques observations nouvelles relatives à l'action photodynamique de l'hématoporphyrine sur le fibrinogène du sang. Le chlorhydrate d'hématoporphyrine cristallisé employé dans mes expériences fut préparé suivant la technique générale indiquée par Mencki et Saleski. Comme les indications qui ont été publiées sur cette méthode ne sont pas très explicites, je rappellerai brièvement le procédé dont je me suis servi :

On prépare de l'hémine d'après la méthode de Mörner (3). Cinq grammes d'hémine sont dissous dans 75 cc. d'acide acétique glacial préalablement saturé d'acide bromhydrique gazeux à 10° C. La solution est maintenue au repos pendant 3 ou 4 jours à la température ordinaire et est ensuite versée dans 600 cc. d'eau. Après repos de plusieurs heures, ce mélange est filtré et, au filtrat, on ajoute de l'hydroxyde de sodium à 10 % jusqu'à formation d'un précipité abondant et décoloration presque complète de la solution. On filtre et on lave le précipité à l'eau jusqu'à ce que l'eau de lavage ne donne plus de réaction avec le nitrate d'argent. Le précipité encore humide est digéré au bain-marie avec de l'hydroxyde de soude dilué à 2 %, dilué avec un égal volume d'eau et filtré. Le filtrat est précipité par l'acide acétique à 33 % que l'on ajoute jusqu'à obtention d'une réaction acide nette. Le précipité est séparé par centrifugation et lavé à l'eau par centrifugation jusqu'à ce que le précipité montre des signes de non-sédimentation. Le résidu humide, obtenu par décantation du liquide surnageant, est traité sur le bain d'eau par l'acide chlorhydrique dilué à 1 %, en évitant avec soin un excès d'acide. La solution est filtrée à chaud pour la séparer de l'abondant résidu gommeux, placée dans un cristallisoir, dans le vide, sur l'acide sulfurique et abandonnée jusqu'à évaporation à sec. Des cristaux se forment. Le résidu est bien lavé avec de l'acide chlorhydrique à 10 %, séché et redissous au bain-marie dans de l'acide chlorhydrique à 10 %, en évitant un excès d'acide. Cette solution est filtrée et le filtrat est additionné d'un dixième de son volume d'acide chlorhydrique concentré. La solution est placée dans le vide sur de l'acide sulfurique jusqu'à cristallisation. Les cristaux sont entraînés par aspiration, lavés avec de l'acide chlorhydrique à 1 %, précipités par addition d'acide chlorhydrique concentré et la solution est de nouveau abandonnée à la cristallisation dans le vide. Le second lot de cristaux, après lavage, est desséché et conservé dans un exsiccateur à l'obscurité.

Les solutions de fibrinogène que j'ai employées dans mes expériences furent préparées à partir du plasma oxalaté de chat, en suivant une modification de la méthode d'HAMMARSTEN, employée pendant un certain temps dans ce laboratoire et dans laquelle la précipitation se fait par le sulfate d'ammonium et non par le chlorure de sodium (4).

Le plasma est précipité par addition d'un volume d'une solution saturée de sulfate d'ammonium à trois ou quatre volumes de plasma. Après centrifugation et lavage, le précipité est dissous dans une solution à 1 % de chlorure de sodium, filtré et reprécipité par addition d'un volume de solution saturée de sulfate d'ammonium à quatre volumes de la solution de fibrinogène. Le précipité est séparé par centrifugation et dissous dans une solution de 1 % de chlorure de sodium, en utilisant la moitié ou les 3/4 du volume primitif de plasma,

En dissolvant le second précipité sans la solution alcaline, il peut être nécessaire d'ajouter quelques gouttes d'une solution de bicarbonate de soude à 1 %. Si on emploie le plasma de chat, deux précipitations suffisent à donner une bonne préparation de fibrinogène; avec le plasma de cheval, trois ou quatre précipitations sont indispensables.

Si, à une solution de fibrinogène préparée de cette façon, on ajoute de petites quantités d'hématoporphyrine et qu'on expose pendant une ou deux heures le mélange à la lumière solaire, ou pendant un temps un peu plus long à une lampe au tungstène (à courte distance), on constatera que le fibrinogène a perdu complètement la propriété de coaguler par la thrombine et a également perdu celle de donner un précipité par la chaleur. Le *modus operandi* de ces expériences fut le suivant :

L'hématoporphyrine fut préparée en solution à 0.2 %, dont 1 à 3 gouttes furent ajoutées à 5 cc. de la solution de fibrinogène. Ce mélange, contenu dans un étroit tube à essai, était placé dans un gobelet d'eau froide et exposé dans la chambre à la lumière directe du soleil. L'eau du gobelet était renouvelée de temps en temps, de façon à maintenir sa température à un niveau égal ou inférieur à 20° C. Dans le même gobelet était placée une solution témoin de fibrinogène sans hématoporphyrine. Une seconde solution témoin de fibrinogène et d'hématoporphyrine était conservée à l'obscurité. Après un temps donné les trois échantillons étaient soumis à la réaction à la chaleur et à la thrombine. Dans le but d'exécuter cette seconde réaction, j'utilisai des échantillons de thrombine sèche, préparée par une méthode décrite antérieurement (5). La thrombine sèche peut être conservée indéfiniment dans un exsiccateur et est facilement soluble dans l'eau ou dans une solution saline diluée.

Des expériences exécutées de cette façon montrèrent que pour les durées indiquées d'exposition à la lumière les solutions de fibrinogène ne sont en rien affectées par la lumière. Elles donnent un caillot gélatineux typique en 3 à 5 minutes avec la solution de thrombine, et le précipité habituel si on les chauffe à 54°-56° C. L'échantillon de fibrinogène et d'hématoporphyrine conservé dans l'obscurité n'est pas modifié non plus après 24 heures ou plus. Il coagule par la thrombine, même plus rapidement que le fibrinogène seul, et est précipité par chauffage à 54°-56° C. Au contraire, l'échantillon de fibrinogène et d'hématoporphyrine exposé à la lumière ne donne aucun caillot avec la thrombine et ne précipite pas par la chaleur entre 60° et 90° C. On peut constater que l'éosine donne des réactions similaires : on utilise une solution d'éosine à 1/1000, dont deux gouttes sont ajoutées à 5 cc. de la solution de fibrinogène.

Si l'exposition à la lumière est raccourcie, des résultats intermédiaires peuvent être obtenus. Ceci signifie que le fibrinogène peut donner un caillot avec la thrombine, mais le temps de coagulation est plus ou moins allongé, ou bien la solution se coagule imparfaitement au lieu de se prendre en une gelée compacte.

Outre ses réactions à la thrombine et à la chaleur, le fibrinogène perd dans ces conditions sa propriété de précipiter par dialyse.

Dans ces expériences, des sacs de collodion fraîchement préparés furent utilisés et l'échantillon de fibrinogène et d'hématoporphyrine qui avait été exposé à la lumière, ainsi qu'un échantillon de fibrinogène seul également illuminé, furent dialysés en présence d'un grand volume d'eau distillée. La solution témoin donna à bref délai un abondant précipité, tandis que la solution à l'hématoporphyrine ne donna ni opalescence ni précipité après une dialyse de nombreuses heures. D'autre part, on constata qu'une solution de fibrinogène et d'hématoporphyrine fut, après exposition à la lumière, précipitée rapidement comme une solution normale de fibrinogène, par addition d'acide acétique dilué ou par une solution saturée de sulfate d'ammonium, ajoutée dans la proportion d'un volume pour quatre ou cinq volumes de la solution.

J'exécutai des expériences du même genre avec de la pseudoglobuline extraite du même plasma, après élimination du fibrinogène qui fut précipité par le sulfate d'ammonium à demi-saturation. La pseudoglobuline fut purifiée par reprécipitation et fut alors, après addition d'hématoporphyrine, exposée à la grande lumière du soleil pendant de nombreuses heures. Même après une exposition prolongée, on constata que la pseudoglobuline ne fut pas altérée en ce qui regarde sa température de coagulation par la chaleur.

L'action photodynamique de l'hématoporphyrine sur le fibrinogène s'exerce plus rapidement quand ce dernier a été purifié par plusieurs précipitations. S'il n'est précipité qu'une fois par le sulfate ammonique, il faut à la lumière un temps plus long pour produire l'effet décrit. Il paraît probable que, dans ce cas comme dans celui de l'action photodynamique de l'éosine sur les érythrocytes, les autres protéines présentes exercent une action protectrice. C'est un fait bien connu que le sérum et de nombreuses protéines protègent les érythrocytes contre l'hémolyse par l'éosine quand elles sont exposées à la lumière. SCHMIDT et NORMAN (6) ont montré que cette action protectrice appartient aussi à quelques amino-acides (tryptophane et tyrosine) et ils pensent que la protection conférée par les protéines est due à la présence de ces amino-acides dans la molécule.

En accord avec cette constatation générale, on trouve que le fibrinogène en plasma oxalaté est moins facilement altéré qu'après précipitation et séparation des autres protéines du plasma. Au cours des observations faites sur le plasma oxalaté, les effets de l'hématoporphyrine et de la lumière furent éprouvés de trois manières : par la chaleur à 56°-60°, par l'action coagulante de la thrombine et par la coagulation causée par recalcification de la solution. En employant du plasma oxalaté de chat additionné d'hématoporphyrine, on trouve qu'une exposition de 4 heures à la grande lumière du soleil est en général suffisante pour empêcher la précipitation du fibrinogène par la chaleur à 60° C. et pour détruire sa propriété de coaguler par addition d'une solution de thrombine. Mais la coagulation causée par recalcification du plasma fut affectée plus lentement et incomplètement. Pour une exposition suffisante, le temps de coagulation par ce procédé fut prolongé depuis 5 à 6 minutes (temps normal montré par le plasma neuf ou le plasma à l'hématoporphyrine conservé à l'obscurité) jusqu'à 45 à 100 minutes. Il est intéressant de noter que SELLARDS a antérieurement publié que des échantillons de plasma oxalaté contenant de la bilirubine ne coagulent pas à la lumière après addition de sérum, bien qu'ils coagulent rapidement après recalcification.

Quelques expériences imparfaites furent entreprises pour déterminer à quelle longueur d'onde la lumière exerce son action photodynamique maximale sur les solutions de fibrinogène et d'hématoporphyrine. Dans une première série, les solutions furent tamisées avec des filtres variés : un filtre rouge laissant passer des longueurs d'onde de 5800 à 6800, un filtre vert laissant passer des longueurs comprises entre 4870 et 5700 et un filtre bleu laissant passer la lumière entre 4950 et 3520. L'effet sur le fibrinogène fut plus rapide sous le filtre vert et moins rapide sous le bleu. Après exposition prolongée, cependant, le fibrinogène montra l'effet habituel dans les trois cas. Dans ces expériences, les tubes contenant le fibrinogène et l'hématoporphyrine furent disposés dans la chambre obscure de telle façon que seule la lumière ayant traversé les filtres pouvait les atteindre. Dans une seconde série, une grille concave de ROWLAND à la longueur focale de 1 mètre ainsi que la lumière d'un arc au fer-carbone furent utilisés. Vu la faible intensité de la lumière, l'action de l'hématoporphyrine sur les érythrocytes fut expérimentée de préférence à son action sur le fibrinogène. Des tubes contenant une suspension à 0.2 % de globules lavés, additionnés chacun de quelques gouttes d'une solution d'hématoporphyrine de 0.2 %, furent exposés au spectre, aux longueurs d'onde de 2400, 3000, 4000, 5000, et au rouge foncé pendant une heure et demie. On utilisa des tubes de quartz pour les échantillons à 2400 et 3000. Pour les temps d'exposition employés, l'hémolyse ne se produisit dans aucun des tubes, mais quand ils furent ultérieurement placés à la lumière solaire l'hémolyse apparut plus rapidement dans le tube préalablement exposé à 5000 et ensuite dans celui préalablement exposé au rouge foncé. Telles qu'elles sont, ces expériences tendent à confirmer les faits observés par HAUSMANN, c'est-à-dire que l'action photodynamique maximale de l'hématoporphyrine se produit avec des longueurs d'onde voisines de 5000, qui correspondent à leur principale bande d'absorption.

A ce propos, j'ai observé une intéressante différence entre l'action des rayons ultraviolets « *rapprochés* » ou « *éloignés* », le dernier terme s'appliquant à des rayons à longueur d'onde inférieure à 3000. La lumière ultraviolette rapprochée fut obtenue en faisant passer la lumière d'un arc à vapeur de mercure à travers du verre ultraviolet de Wood, qui a une longueur de transmission intermédiaire entre 3900 et 3300. Une telle lumière est sans effet (pour le temps d'exposition utilisé) sur des solutions de fibrinogène seul, mais sur les solutions qui contiennent de l'hématoporphyrine le fibrinogène, après une exposition de 1 à 2 heures, montre les effets décrits ci-dessus, notamment une perte complète de la propriété de précipiter par la chaleur ou de coaguler par la thrombine. Les effets des rayons ultraviolets éloignés furent obtenus en exposant dans des tubes de quartz les solutions à la lumière non tamisée d'un arc à vapeur de mercure. Cette lumière cause la précipitation des solutions de fibrinogène après une exposition de 1/2 à 3/4 heure, et ceci s'accorde avec son action coagulante bien connue sur des solutions de protéine. Quand de l'hématoporphyrine était ajoutée à la solution de fibrinogène, cette précipitation ne se produisait pas. La solution restait claire, mais elle ne donnait plus de caillots avec la thrombine.

Plusieurs auteurs ont supposé que l'action sensibilisante de l'hématoporphyrine et d'autres réactifs photodynamiques consiste essentiellement dans une altération de la substance considérée, telle qu'elle rend les longues ondes lumineuses capables d'effectuer les changements que l'on sait être causés par les courts rayons ultraviolets.

Schanz (8) a établi que la lumière agit sur les solutions de protéines en les rendant moins solubles, ainsi que l'indique la plus grande facilité de précipitation, et que cet effet est facilité par l'éosine, l'hématoporphyrine et d'autres sensibilisateurs. Cette conclusion n'est évidemment pas applicable au cas du fibrinogène.

Tandis que les rayons ultraviolets éloignés causent la précipitation du fibrinogène, cette réaction est empêchée par la présence de l'hématoporphyrine. *L'effet de ce sensibilisateur est de rendre les lumières visible et ultraviolette capables de changer le fibrinogène en une forme plus soluble de protéine. Le fibrinogène, insoluble dans l'eau, est changé en une protéine soluble dans l'eau, protéine non précipitée par la chaleur et non coagulée de ses solutions par l'action de la thrombine. Cette modification est analogue à celle que produit l'action des alcalis.*

La façon dont l'hématoporphyrine participe à la réaction n'a pas encore été découverte. On sait parfaitement que pour que la réaction photodynamique se produise, l'hématoporphyrine et la substance sur laquelle elle agit doivent être en contact intime l'une avec l'autre pendant que la lumière agit. Une exposition préalable de l'hématoporphyrine à la lumière ne détermine pas de changement capable d'affecter le fibrinogène quand les solutions sont ultérieurement mises en contact dans l'obscurité, et une solution d'hématoporphyrine séparée par une mince lame de quartz d'une solution de fibrinogène n'exerce aucune action photodynamique si on l'expose à la lumière. Il semblerait que l'hématoporphyrine déclanche ou facilite l'action de la lumière à la façon d'un catalyseur.

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AN ARTIFICIAL NERVE

PHYSIOLOGISTS are keenly interested in all attempts to discover an explanation or an analogy for the passage of the nerve stimulus. Most enlightening suggestions have recently been presented by Lillie[1] in his studies of passivity phenomena in pure iron wires. It seems that the transmission of the momentary wave of activity which occurs in a passive iron wire on activation in 70% nitric acid is closely analogous both chemically and electrically to the passage of the nerve impulse.

The general similarity of the two phenomena was apparently first noticed by Wilhelm Ostwald and subsequently elaborated by his student Heathcote.[2] In a paper published in 1907 under the caption "Transmission along a nerve" (p. 909) Heathcote writes as follows:

> In 1900, then, Prof. Ostwald called our attention to the possibility of nerve transmission being a process akin to the transmission of activity. . . . It is to be expected . . . that transmission of activity would be slower immediately after the first transmission owing to products of reaction around the iron. This has been confirmed by direct experiments in the case of iron in nitric acid. An effect of this kind in a nerve would explain the nature of "fatigue" so far as it concerns nerves.

After discussing the small amount of energy consumption in both transmissions Heathcote summarizes his conclusions as follows:

> There is nothing in the structure of nerve which renders it impossible to regard transmission as

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wire registering a diphasic "action current" on the galvanometer.

In its passage down the spring the activation wave sets free a shower of minute bubbles which change the color of the acid sufficiently to make the wave of activity clearly visible even at some distance from the preparation. This preparation recovers rapidly at room temperature and may be used repeatedly to demonstrate mechanical, chemical and electrical stimulation as well as the time required for the passage of a single activation wave over a distance of ten meters. At the close of the demonstration the coil should be removed from the burette, thoroughly rinsed in slightly alkine water and alcohol and rubbed briskly with a rough cloth. With these precautions it may be used repeatedly.

REYNOLD A. SPAETH

THE PHYSIOLOGICAL LABORATORY,
SCHOOL OF HYGIENE AND PUBLIC HEALTH,
JOHNS HOPKINS UNIVERSITY

The passage of the wave of activation over the surface of a short wire is so rapid, that it is not easily demonstrated to a large group of students. The simple arrangement here described is clearly visible at a considerable distance and has been used successfully as a lecture table demonstration.

Nine and a half meters of a ten-meter piece of number 20 piano wire are wound by hand on a machine lathe into a spring small enough to slip easily into a 100 c.c. burette. After stretching the spring sufficiently to insulate the individual turns, a glass tube is inserted in the spring and the remaining half meter of wire is returned through this tube. When set into the burette the upper end of this tube should just reach the burette top (Fig. 1). The two free ends of the piano wire are now connected through thin iron wires with a demonstration galvanometer or voltmeter which registers both positive and negative variations. After filling the burette three quarters with 70 per cent. c.p. nitric acid (by volume) the spring coil is lowered into it until about an inch of the lower end of the coil is submerged in the acid. The submerged inch of wire immediately begins to dissolve but if the coil is held in this position until chemical action ceases, the entire wire may be lowered into the acid without further action. In other words by passifying one end of a wire and then slowly lowering the remainder of that wire into acid the entire piece is passified. To prevent activation the wire must be lowered slowly and steadily. The coil is now ready to be tested at intervals with a zinc or copper "stimulus" applied just at the surface of the nitric acid at the top of the burette. After a somewhat variable latent period the entire spring becomes activated. The wave of activation passes down the coil and back through the return wire registering a diphasic "action current" on the galvanometer.

In its passage down the spring the activation wave sets free a shower of minute bubbles which change the color of the acid sufficiently to make the wave of activity clearly visible even at some distance from the preparation. This preparation recovers rapidly at room temperature and may be used repeatedly to demonstrate mechanical, chemical and electrical stimulation as well as the time required for the passage of a single activation wave over a distance of ten meters. At the close of the demonstration the coil should be removed from the burette, thoroughly rinsed in slightly alkine water and alcohol and rubbed briskly with a rough cloth. With these precautions it may be used repeatedly.

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JOHNS HOPKINS UNIVERSITY

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THE RELATION BETWEEN FATIGUE AND THE SUSCEPTIBILITY OF RATS TOWARDS A TOXIN AND AN INFECTION.*

BY ELLA HUTZLER OPPENHEIMER AND REYNOLD A. SPAETH.

(Received for publication, October 1, 1921.)

PRELIMINARY.

There is a popular belief in the medical world today that a fatigued individual is more susceptible to disease than a non-fatigued individual. There are no available statistics to prove or disprove this opinion. All that is known is that workers in industry, and they necessarily must be included in the group of " fatigued individuals," become infected as do the idle onlookers. There are no comparative figures on the susceptibility rates for the two groups.

This series of experiments attempts to discover whether a state of fatigue will produce in experimental animals a greater susceptibility to toxins and infections. By the term " fatigue " we mean a condition of temporary exhaustion produced by excessive muscular exertion. In our work with tetanus toxin, we found that while fatigue did not markedly reduce susceptibility, neither did it increase it in white and hooded rats. There was, however, a definite tendency for fatigue to increase the resistance of these rats towards an infection of pneumococci.

PREVIOUS WORK ON FATIGUE AND SUSCEPTIBILITY.

Although we found no record of any work on the relation between fatigue and susceptibility to toxins, we discovered in the literature accounts of similar work by two groups of investigators on the influence of fatigue on susceptibility to infections. Their results were not the same.

In 1890, Charrin and Roger (1) (2) injected 22 white rats with anthrax bacilli of different virulence. Of the 11 rats injected with " charbon bacteridien," 3 unfatigued controls all survived. The other 8 rats were fatigued after injection. One of these survived; 2 died within 24 hours; the other 5 died after 3 days. Eleven rats were injected with "charbon symptomatique." The 5 controls all lived. The 6 rats fatigued after injection all died; 3 within 24 hours, 2 others after 30 hours and 1 after 50 hours. These results indicate that fatigue after the injection of anthrax bacilli favors the development of this infection in the white rat.

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* From the Physiological Laboratory, School of Hygiene and Public Health, Johns Hopkins University.

In 1910, Abbot and Gildersleeve (3) fatigued 18 rabbits and found that there was always a reduction of the opsonic index. From this it was expected that the susceptibility to the pyogenic organisms would be heightened, but the results were indefinite. Of 7 fatigued rabbits injected intravenously with *Streptococcus pyogenes*, 6 died of fatal septicemia from 6 to 30 days earlier than the controls. The seventh rabbit died at the same time as the control. The results with *Staphylococcus pyogenes* were entirely negative. Eleven exhausted animals with lessened opsonizing powers did not react differently from the controls when inoculated intravenously or subcutaneously.

These results, in so far as they are positive, confirm the generally accepted conception of the relation between fatigue and "natural susceptibility."

EXPERIMENTAL.

The animals with which we worked were rats, white and hooded. In spite of the fact that the M.L.D. determinations for both tetanus and diphtheria toxins had been made for guinea pigs, we substituted the rats because of the ease with which they run and can be fatigued. The size of the rats was an additional advantage.

All of our rats were vigorous, healthy animals and throughout the period of experimentation no abnormalities of any kind appeared in the stock. None succumbed to extraneous infections. The rats were fed once daily a uniform McCollum diet of the following formula:

3 parts oat meal
3 parts whole wheat flour
3 parts corn meal
1 part flax-seed meal.

PRELIMINARY EXPERIMENTS WITH TETANUS TOXIN.

In the first experiment we tried to discover whether fatigued rats are more susceptible to tetanus toxin than normal, healthy rats of the approximate age and size and under the same surrounding conditions of temperature, humidity and light. Before this could be determined the M.L.D. of tetanus toxin had to be established for white and hooded rats.*

From information obtained from the literature and other sources, we were led to believe that rats are highly resistant to all toxins including tetanus toxin. But as the rats are such ideal animals for exercising and fatiguing, we attempted the M.L.D. determinations for the tetanus toxin even though it appeared to be unfavorable. Therefore, as the appended table shows, we began by giving the rats large doses of the toxin and decreased the doses gradually. The M.L.D.—that is, the dose of toxin that would kill a rat within a week—was 0.00,000,004 gr. toxin per gram weight of rat. For a 150 gr. rat, therefore, the dose was 0.00,000,6 gr. toxin. The lethal dose, however, is much less, if no time limit be placed upon death. In our experiments we used a just sublethal dose and although this did not kill, it usually paralyzed the rat. In some cases we used a just lethal dose so that the effect of the fatigue, if there were any, could be more plainly seen.

Table I in the Appendix shows the effect of the tetanus toxin in the determination of its M.L.D. On the basis of these determinations, we decided to use doses of 0.00,000,002,6 and 0.00,000,002,4 gr. tetanus toxin per gram weight of rat for the fatigue experiments. The toxin after being weighed was dissolved in normal saline solution and injected subcutaneously.

APPARATUS.

The apparatus used for fatiguing the rats was essentially similar to that of Richter (4). It consisted of two sets of wire drums, three drums in each set. The drums were made of one quarter inch mesh galvanized wire net. The dimensions and details of a single drum are shown in Fig. 1. Each drum was mounted on a bicycle hub. This we found necessary since, when an axle ran through the center of the drum, the rats would perch upon this axle instead of running. The total distance run was recorded on each drum separately by a Veeder cyclometer arranged with a trip lever as shown in the figure. At the front of each drum, there was a heavy drop door lined with zinc and held in place by a spring catch. The circular one half inch oak back

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* We are indebted to Dr. George W. McCoy for the toxin used in these experiments, and to Dr. J. P. Leake for the method of handling the toxin.

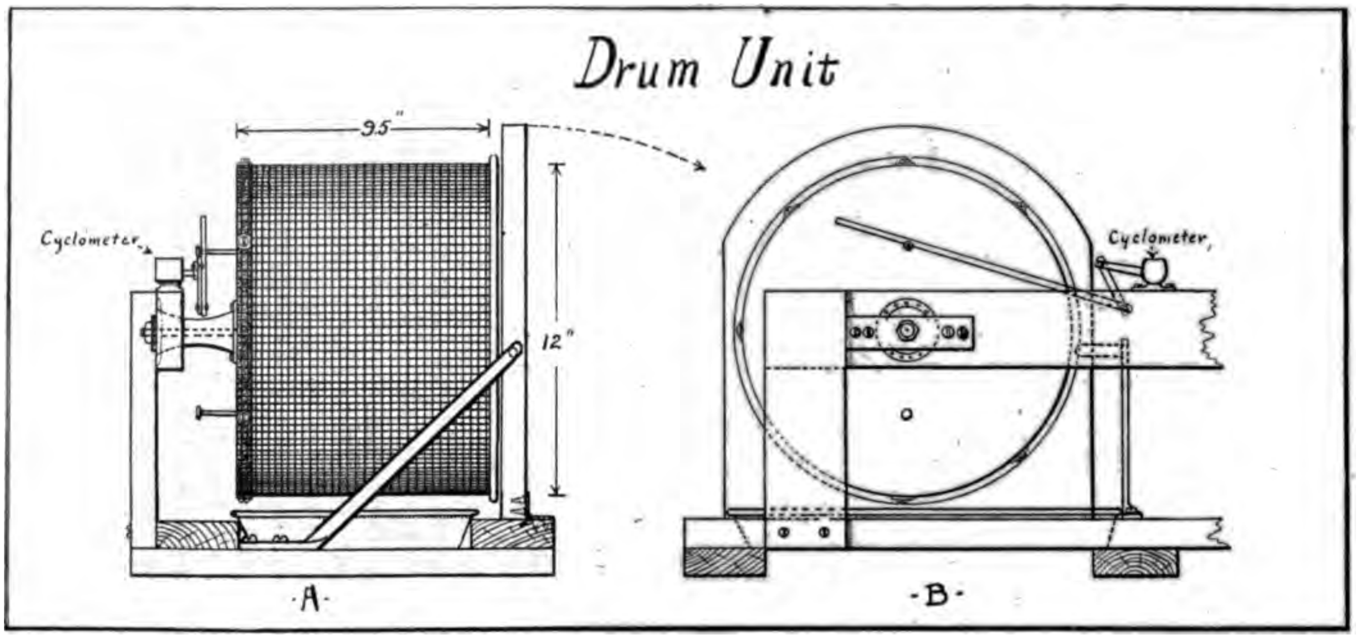

FIG. 1. *A*, side, and *B*, rear view, of a drum unit used in fatiguing the rats. Six drums in two sets of three each were used in the experiments. Heavy canvas webbing, one and a quarter inches wide, was used as belting. The drums themselves acted as pulleys. The frame was made of one inch redwood.

of each drum was likewise lined with zinc. The complete set-up consisted of six drums belted in parallel, two sets of reducing pulleys, a motor, and rheostat.

METHOD OF FATIGUING ANIMALS.

We attempted at first to make the rats spontaneously fatigue themselves in these drums as in a treadmill. But while the moving drum is actually a stimulus which serves to keep the rats running, we could not count on an end result of fatigue. Following Richter (loc. cit.), rats were run for a series of days, 6 hours at a time with 6 hours of rest intervening. At first their activity was slight but it gradually increased, following a regular learning curve. After a few trials, they would run as much as 4 miles in the 6-hour interval, but before there was any evidence of fatigue, they would spontaneously rest. Thus, the rats, instead of becoming exhausted by fatigue, improved their physical condition by this form of exercise.

In fatiguing the rats, therefore, the drums were turned by an electric motor. Under these conditions spontaneous rest was practically eliminated. By a series of pulleys and a rheostat, we could regulate the speed so that a definite distance would be covered in a given interval. When the rats fought against the revolving drum, they were quickly fatigued. Exhaustion came less rapidly when the drum was turned at a slow rate for a long time than when it revolved rapidly for a short time. In most cases as fatigue became pronounced, the rats barely moved one foot after another and as soon as the drum ceased moving, they immediately fell asleep.

MEASUREMENT OF FATIGUE.

The entire distance covered by the rats could be computed by multiplying the circumference of the drum by the number of revolutions. We attempted at first to run each rat for the same time and over the same distance, hoping that the fatigue resulting would be approximately the same. But this was not the case. The greater the ease with which a rat ran, the longer it took to become fatigued.

Therefore, in fatiguing the rats, the result looked for was not fatigue in terms of distance or time run, but in terms of the effect on the rat and its physical state. When it was so greatly fatigued that it no longer ran, was carried about by the drum, and, in general, failed to respond to auditory or tactile stimuli and was content merely to lie and rest, we considered the rat exhausted and completely fatigued (5). Our criteria of fatigue were, therefore, purely empirical and qualitative.[*]

Although the rats lost weight whenever they reached this stage, the condition was not lasting. This was proved by running them on several succeeding days. After running for 4 hours on one day, they usually had completely recovered their normal weight before running again the next day. Therefore, the rats were not permanently harmed, but were, indeed, only fatigued.

ADJUSTMENT OF RUNNING PERIODS AND INJECTIONS.

At first, we fatigued the animals once immediately before injection. As this seemed to have no effect on the rats' susceptibility, we increased the amount of fatigue by exhausting the rats in three successive periods. They were fatigued two days before, the day before, and immediately preceding the injection. Other rats were injected before being fatigued. In the case of the tetanus toxin, we assumed that unless fatigue occurred after the latent period of the toxin had passed, the effect would be approximately the same as fatigue before injection. Therefore, the rats were fatigued not only immediately after receiving their dose of the toxin, but also on the two succeeding days. In this way, both toxin and fatigue could be effective as the latent period of the former was surely completed.

At least 3 rats were injected at the same time in each experiment. One rat served as a control. It was injected with the same dose as the rat fatigued before injection. A third rat was injected and subsequently fatigued as described above. The general experimental scheme was, therefore, as follows:

Group 1. Rested ....injected........rested (control)
Group 2. Fatigued ......injected........rested
Group 3. Rested ....injected........fatigued

EFFECTS OF FATIGUE ON SUSCEPTIBILITY TO TETANUS TOXIN.

In the case of tetanus toxin the variation in the susceptibility of fatigued and normal rats was slight and was not always in the same direction.

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[*] In a few cases, the rats discovered that by lying on their backs and raising their feet, the drum would slip by beneath them without seriously disturbing their position. When violent tapping on the cage no longer made these animals jump to their feet and begin running, they were considered exhausted.

Of the 22 rats injected, none of the 8 animals fatigued before injection died before the controls; 1 was affected in the same way as the control, and 2 of these rats were paralyzed more severely than the controls and were slower in recovering. Of the 8 rats fatigued after injection, 1 died before the control; 4 survived the controls; 1 died at the same time; 1 was paralyzed more severely and took longer to recover than did the control, and 1 was paralyzed more severely but recovered at the same time.

Fatigue, therefore, increased the susceptibility of 6 rats (37 per cent.), increased the resistance of 9 rats (56 per cent.), and failed to affect 1 rat (6 per cent.). Although there are too few cases to warrant any very positive conclusions, we were nevertheless struck by the fact that fatigue apparently increased the resistance rather than the susceptibility of the majority of our animals.*

The experimental results will be found in detail in Table II of the Appendix.

RELATION BETWEEN FATIGUE AND PNEUMOCOCCAL INFECTION.

We selected Type I pneumococcus as a suitable organism for testing the relation between fatigue and infection. We used 18-hour broth cultures of a virulent organism and diluted it to the desired strength with sterile broth. We made intraperitoneal injections.†

We continued using white and hooded rats as we found these animals to be most satisfactory. We used the same apparatus as in the tetanus toxin experiments and the animals were fatigued in the same way. That is, we considered an animal exhausted when it could no longer run and rolled on its back and was carried about by the drum—whether it had run 1 hour or 6, or had covered 1 or 4 miles. Some of the rats we fatigued once before injection; some were fatigued three times before injection; some were fatigued once after injection, and others in three periods after having been injected.

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* We desired to use another toxin to discover whether we could get comparable results. We, therefore, attempted to find the M.L.D. of diphtheria toxin for white and hooded rats. In this case, however, so great a dose is necessary to kill a rat that we were unable to determine it. In smaller doses, up to 2 c.c., the toxin affected the rats quite like injections of a similar amount of normal saline solution.

† In the course of the work with pneumococcus, an observation was made that seems worth recording. Eleven rats developed a pathological condition of the eye from the injection of the pneumococci. In two cases, both eyes became completely opaque; the others were affected in only one eye. In only two of the eleven rats did the eyes clear up. The autopsies showed that in no case was the lens cloudy, the opacity being limited to the cornea.

Seventy-eight rats were injected; 18 of these were unfatigued controls and the other 60 rats were fatigued. In this case, a clear-cut result was obtained. Fatigue not only failed to lower the rats' resistance to the pneumococci, but actually decreased their susceptibility to this infection (see Fig. 2).

Of the rats fatigued before injection, 26 survived the controls; 1 died before the control; 1 died simultaneously with the control, and 2 lost weight and recovered with the controls. Of the rats fatigued after being injected, 15 survived the controls; 7 died before the controls; 5 died at the same time as their controls, and 3 lost weight and recovered with their controls. In other words, fatigue increased the resistance of 67 per cent. of the rats, and only increased the susceptibility of 13 per cent. The susceptibility of 20 per cent. was unaffected.

As a concluding experiment in the above series (Series C, Experiment 11), we injected 17 rats—6 we used as controls, 6 we fatigued before injection and 5 we fatigued after injection. Five of the 6 controls died in 3 to 6 days; the 6 rats fatigued before injection all survived and 3 of the others died in 4 to 5 days. That is, 5 out of 6, or 86 per cent. of the control rats died, and only 3 of 11, or 27 per cent. of the fatigued rats died. This experiment definitely confirmed our earlier results showing an increased resistance to pneumococcal infection produced by fatigue. Fig. 2 shows the results of experiments 9, 10 and 11, Series C. In every case the rat received 0.0002 c.c. of a culture that had been transplanted twice. All rats below the zero line lived: all above, indicated by black rectangles, died.

A study of Table III in the Appendix shows that fatigue *before* the injection of pneumococci is nearly twice as potent (86.6 per cent.) in raising the resistance to this infection as is fatigue which follows the pneumococcal injection (50 per cent.). We have no explanation to offer at present either for the general fact of increased resistance resulting from fatigue or for the greater resistance produced by fatigue which precedes the injection. Further experiments, now in progress, may throw some light on a physical-chemical mechanism that will account for the phenomena.

GENERAL CONCLUSIONS.

1. The susceptibility of white and hooded rats to tetanus toxin is far greater than has hitherto been supposed. The M.L.D. is 0.00,000,004 gr. toxin per gram weight of rat.

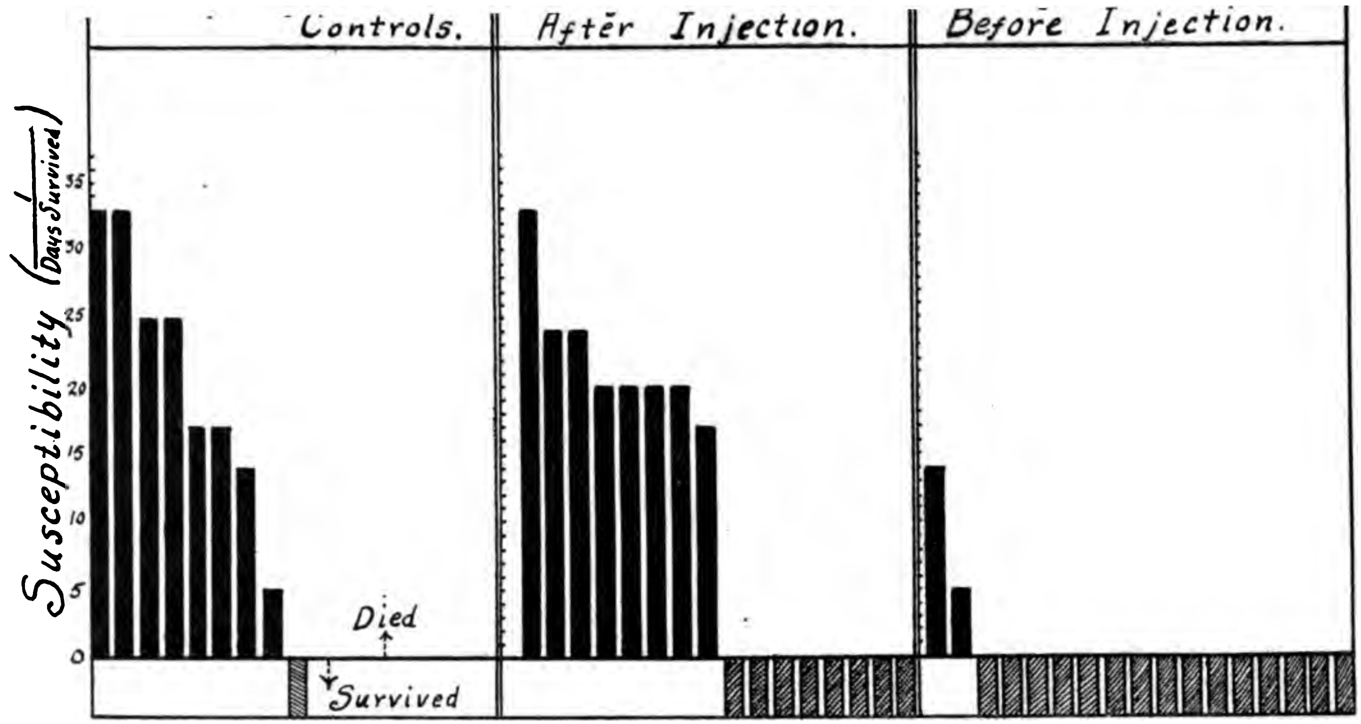

FIG. 2. A composite chart showing the relation between fatigue and susceptibility to pneumococcal infection in the forty-two rats of Experiments 9, 10, and 11, Series C. Each black rectangle represents a single rat that died from an injection of 0.0002 c.c. of our virulent strain of pneumococcus. The cross-hatched rectangles below the zero line represent rats that survived the experiment; i.e., rats that lived indefinitely after completely recovering from the injection. In such cases the susceptibility coefficient $\frac{1}{\text{(days survived)}}$ was assumed to equal zero.

2. Fatigue, artificially induced in white and hooded rats by forcing them to run in motor-driven drums, apparently tends slightly to increase their resistance to subcutaneous injections of tetanus toxin. This occurs whether fatigue precedes or follows the injection of the toxin.

3. White and hooded rats, up to 185 grams in weight, are not susceptible to diphtheria toxin in doses as large as 2 c.c.

4. Fatigue, both preceding and following intraperitoneal injections of Type I pneumococcus, definitely increases the resistance of the white and hooded rats to that infection.

5. At least in the case of white and hooded rats, our results contradict the popular belief that a fatigued individual is more susceptible to disease (tetanus toxin and pneumococcus infection) than a non-fatigued individual.

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APPENDIX.

TABLE I.

SERIES A.

The M.L.D. of tetanus toxin for white and hooded rats.

Rat No.	Wt. of rat in gms.	Dose toxin in grams $\times 10^{-6}$.	Amt. toxin per gm. wt. rat $\times 10^{-7}$.	Injected.	Paralysis began.	Recovery.	Death.
1.....	135	10,000	740	Oct. 28th	—	—	2d day
2.....	155	5,000	320	"	—	—	"
3.....	170	2,500	140	"	—	—	"
4.....	205	2,500	120	"	—	—	"
5.....	205	1,000	48	"	—	—	"
6.....	140	500	35	Nov. 2d	2d day	—	"
7.....	210	500	23	Oct. 28th	"	—	3d day
8.....	139	250	18	Nov. 2d	"	—	2d "
9.....	166	125	7.5	"	"	—	3d "
10.....	153	31	2.0	"	"	—	"
11.....	154	25	1.6	Nov. 5th	"	—	"
12.....	163	17.5	1.1	"	3d day	—	4th day
13.....	103	7.0	.679	Dec. 1st	"	—	8th "
14.....	205	10.0	.486	"	"	—	4th "
15.....	120	6.0	.486	"	2d day	--	"
16.....	110	5.0	.486	"	"	—	6th "
17.....	145	7.0	.486	Dec. 7th	3d day	—	"
18.....	107	5.0	.486	"	2d "	—	4th day
19.....	181	8.75	.483	Nov. 5th	3d "	—	20th "
20.....	193	7.7	.400	Dec. 7th	"	—	5th ."
21.....	145	5.8	.400	"	"	—	"
22.....	115	4.6	.400	"	4th day	—	7th day
23.....	139	5.0	.360	Dec. 14th	6th "	24th day	—
24.....	124	4.5	.360	Jan. 5th	5th "	—	11th day
25.....	123	3.7	.300	Dec. 14th	6th "	—	7th "
26.....	115	3.2	.280	Jan. 17th	3d "	—	6th "
27.....	129	3.3	.260	Jan. 31st	4th "	—	5th "
28.....	116	2.7	.250	Dec. 14th	9th "	17th day	—
29.....	125	3.1	.250	Jan. 5th	—	—	—
30.....	166	4.15	.250	Jan. 24th	8th day	32d day	—
31.....	167	4.20	.250	Feb. 9th	2d "	—	4th day
32.....	96	2.40	.250	Feb. 25th	3d "	—	20th "
33.....	124	2.90	.240	Feb. 16th	7th "	10th day	—
34.....	108	2.30	.214	Dec. 14th	4th "	—	20th day
35.....	215	4.60	.214	Jan. 5th	--	—	—
36.....	98	1.80	.180	"	5th day	12th day	—
37.....	119	1.80	.150	"	—	—	—

TABLE II.

SERIES B.

The relation between fatigue and susceptibility to tetanus toxin in white and hooded rats.

Exper. No.	Wt. of rat in gms.	Dose toxin in grams $\times 10^{-6}$.	Amt. toxin per gm. wt. rat $\times 10^{-8}$.	Date of injection.	Fatigued.			Paralysis began.	Recovery complete.	Death.	Susceptibility.
					Number of times.	Before inject.	After inject.				
1	120	3.0	2.5	Jan. 17th	1	–	×	2d day	32d day	–	–
	120	3.0	2.5	"	1	×	–	"	"	–	–
	112	2.8	2.5	"	0	–	–	"	"	19th day	– – – –
2	148	3.6	2.5	Jan. 24th	1	×	–	2d day	32d day	–	±
	164	4.1	2.5	"	1	–	×	"	"	–	±
	166	4.15	2.5	"	0	–	–	8th day	"	–	– – – –
3	138	3.6	2.6	Jan. 31st	1	×	–	2d day	–	6th day	–
	150	3.9	"	"	1	–	×	"	–	5th "	±
	145	3.8	"	"	1	–	×	4th day	–	7th "	–
	129	3.3	"	"	0	–	–	"	–	5th "	– – – –
4	127	3.2	2.5	Feb. 9th	3	–	×	3d day	–	5th day	–
	136	3.4	"	"	3	–	×	"	–	6th "	–
	125	3.1	"	"	3	×	–	2d day	–	5th "	–
	117	3.0	"	"	3	×	–	"	–	5th "	–
	167	4.2	"	"	0	–	–	"	–	4th "	– – – –
5	177	4.2	2.4	Feb. 16th	3	–	×	5th day	19th day	–	+
	131	3.1	"	"	3	×	–	"	12th "	–	+
	141	3.4	"	"	3	×	–	"	19th "	–	+
	124	2.9	"	"	0	–	–	7th day	10th "	–	– – – –

The relation between fatigue and susceptibility of rats towards injections of Type I pneumococcus.

Exper. No.	Wt. of rats in gms.	Dose in c.c. of Type I pneumococcus.	No. of culture transplants.	Date of injection.	Fatigue.			Death.	Susceptibility.
					Number of times.	Before inject.	After inject.		
1	126	0.0002*	4	April 2d	1	–	×	22d day	+
	126	"	"	"	2	×	–	–	±
	126	"	"	"	0	–	–	–	– – – –
2	134	0.002	4	April 2d	1	–	×	–	±
	115	"	"	"	2	×	–	24th day	+
	119	"	"	"	0	–	–	–	– – – –
3	113	0.02 norm. saline	– – – –	April 8th	1	×	–	–	– – – –
	113	0.02	5	"	1	×	–	–	±
	105	"	"	"	1	–	×	–	±
	122	"	"	"	1	–	×	–	±
	120	"	"	"	0	–	–	–	– – – –
4	111	0.02	1	April 15th	1	×	–	5th day	–
	137	"	"	"	1	×	–	9th "	–
	118	"	"	"	1	–	×	2d "	±
	109	"	"	"	1	–	×	10th "	–
	110	"	"	"	0	–	–	2d "	– – – –
5	123	0.02	2	April 22d	3	×	–	5th day	–
	120	"	"	"	1	×	–	4th "	–
	138	"	"	"	1	–	×	–	–
	150	"	"	"	1	–	×	4th day	–
	123	"	"	"	0	–	–	3d "	– – – –

* 18-hour broth culture.

TABLE III. ***Series C*** (*Continued*).

Exper. No.	Wt. of rats in gms.	Dose in c.c. of Type I pneumococcus.	No. of culture transplants.	Date of injection.	Fatigue.			Death.	Susceptibility.
					Number of times.	Before inject.	After inject.		
6	124	0.02	3	April 29th	2	×	–	7th day	–
	135	"	"	"	2	×	–	–	–
	156	"	"	"	1	–	×	6th day	–
	119	"	"	"	1	–	×	4th "	±
	117	"	"	"	0	–	–	4th "	----
7	92	0.002	3	May 4th	3	×	–	6th day	–
	102	"	"	"	3	×	–	–	–
	123	"	"	"	3	–	×	–	–
	119	"	"	"	3	–	×	5th day	–
	119	"	"	"	0	–	–	3d "	----
8	97	0.0002	3	May 11th	3	×	–	–	–
	114	"	"	"	3	×	–	–	–
	175	"	"	"	3	–	×	3d day	+
	140	"	"	"	3	–	×	7th "	–
	158	"	"	"	0	–	–	4th "	----

TABLE III. *Series C (Continued).*

Exper. No.	Wt. of rats in gms.	Dose in c.c. of Type I pneumococcus.	No. of culture transplants.	Date of injection.	Fatigue.			Death.	Susceptibility.
					Number of times.	Before inject.	After inject.		
9	92	0.0002	2	May 25th	3	×	–	–	–
	122	"	"	"	3	×	–	–	–
	98	"	"	"	3	×	–	–	–
	109	"	"	"	3	×	–	–	–
	100	"	"	"	3	×	–	–	–
	158	"	"	"	3	–	×	–	–
	151	"	"	"	3	–	×	–	–
	163	"	"	"	3	–	×	–	–
	134	"	"	"	3	–	×	5th day	+
	149	"	"	"	3	–	×	4th "	+
	168	"	"	"	3	–	×	6th "	+
	126	"	"	"	0	–	–	19th "	----
	123	"	"	"	0	–	–	6th "	----
10	114	0.0002	2	June 1st	3	×	–	–	–
	94	"	"	"	3	×	–	–	–
	112	"	"	"	3	×	–	–	–
	94	"	"	"	3	×	–	–	–
	87	"	"	"	3	×	–	7th day	±
	90	"	"	"	3	×	–	19th "	–
	105	"	"	"	3	–	×	–	–
	127	"	"	"	3	–	×	–	–
	120	"	"	"	3	–	×	–	–
	117	"	"	"	3	–	×	3d day	+
	122	"	"	"	3	–	×	5th "	+
	143	"	"	"	0	–	–	7th "	----

TABLE III. *Series C* (*Continued*).

Exper. No.	Wt. of rats in gms.	Dose in c.c. of Type I pneumococcus.	No. of culture transplants.	Date of injection.	Fatigue. Number of times.	Fatigue. Before inject.	Fatigue. After inject.	Death.	Susceptibility.
11	90	0.0002	2	June 8th	3	×	–	–	–
	112	"	"	"	3	×	–	–	–
	85	"	"	"	3	×	–	–	–
	80	"	"	"	3	×	–	–	–
	82	"	"	"	3	×	–	–	–
	82	"	"	"	3	×	–	–	–
	122	"	"	"	3	–	×	–	–
	152	"	"	"	3	–	×	–	–
	124	"	"	"	3	–	×	5th day	±
	118	"	"	"	3	–	×	"	±
	152	"	"	"	3	–	×	4th day	±
	95	"	"	"	0	–	–	6th "	– – – –
	102	"	"	"	0	–	–	3d "	– – – –
	97	"	"	"	0	–	–	3d "	– – – –
	90	"	"	"	0	–	–	4th "	– – – –
	170	"	"	"	0	–	–	4th "	– – – –
	104	"	"	"	0	–	–	–	– – – –

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THE ACTION OF ULTRAVIOLET LIGHT ON EGG ALBUMIN IN RELATION TO THE ISOELECTRIC POINT

JANET H. CLARK

From the School of Hygiene and Public Health, Johns Hopkins University

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Ultraviolet light of wavelength less than 300 or 330 $\mu\mu$ produces marked physiological effects. Light filtered through glass, containing only wavelengths greater than 330 $\mu\mu$, has no effect except in the presence of sensitizers. A number of observers have reported that one of the results of ultraviolet radiation is the coagulation of egg albumin (1), (2), (3). Burge (4) found that lens protein coagulates less easily than egg albumin. Pincussen (5), in reviewing the subject of light, says that colloids generally tend toward greater aggregation under ultraviolet light.

In a recent review (6) I have proposed a general theory of light action which may be summarized as follows. It is known that absorption of ultraviolet light, of wavelength less than 300 $\mu\mu$, usually results in the emission of electrons, so that a negatively charged substance loses its charge and an uncharged substance acquires a positive charge. This phenomenon is called the photo-electric effect. The number and velocity of these emitted electrons increase with decreasing wavelength of the exciting light (7), (8). For each substance there is a long wavelength limit to the effect, or a photo-electric threshold, so that all wavelengths shorter and no wavelengths longer than this threshold wavelength stimulate the emission of electrons. The threshold is fixed for every substance under given conditions, but is very subject to shifts with changes in surface conditions. The photo-electric effect is especially marked in metals, where ultraviolet light is absorbed in a surface film, but it is found in most solids and also in a great many liquids. In liquids, however, little light is absorbed in a surface layer so that few electrons are lost from the surface and the photo-electric effect becomes latent, the emission of electrons by atoms in the interior being usually shown by an increase in conductivity.

I have supposed that the first step in all the actions of light on organic substances is an emission of electrons from the substance affected, that is, the ionization of photo-electric molecules. This state of ionization may be likened to the latent image in a photographic plate and subsequent development of this latent image by chemical action leads to the various physiological light effects. In other words, the physiological actions of light are due to the formation of photochemical compounds, and the formation of these compounds is due to the photo-electric action of light which, by producing an ionized condition in light sensitive molecules, leads to subsequent chemical reactions. The photodynamic sensitization of substances, so that they react toward visible light, in the presence of certain dyes, in the same way that the untreated substance acts toward ultraviolet light, is explained thus. In the presence of a sensitizer the photo-electric threshold of the light-sensitive substance is shifted toward longer wavelengths, so that it is now photo-electric at these wavelengths and is therefore ionized, with resultant chemical action, by visible light.

To make this theory tenable it must first be proved that the effect of ultraviolet light is due to the emission of electrons from light-sensitive atoms and molecules. Secondly, it must be proved that this emission takes place in visible, as well as in ultraviolet light on the addition of a sensitizer. The second part of the proof is still wanting, but the results of this paper, on the coagulation of egg albumin by ultraviolet light, furnish excellent evidence for the first part.

Suppose, as a premise, that the absorption of ultraviolet light by the particles of colloidal egg albumin results in the emission of electrons. These colloidal particles are known to have no charge at the isoelectric point (pH 4.8), a negative charge on the alkaline of the isoelectric point (pH greater than 4.8), and a positive charge on the acid side (pH less than 4.8). On radiating with ultraviolet light less than 330 $\mu\mu$ one would therefore expect the following results. In solutions with pH greater than 4.8 the particles should lose their negative charge, and some would acquire a positive charge, precipitating the others as coagulated albumin. In these solutions radiation would lead to greater aggregation. For pH = 4.8, the particles being uncharged would, on radiation, acquire a positive charge and repel each other. This would give greater dispersion. For pH less than 4.8 the particles have a positive charge to begin with and, on radiation, this charge would become greater, giving greater dispersion.

If radiated through glass there should be no change in the solutions, unless a sensitizer is present, since egg albumin is not photo-electric for wavelengths greater than 330 $\mu\mu$. That egg albumin is photo-electric for wavelengths less than 330$\mu\mu$ has been shown by Schanz (9) and has also been confirmed in this laboratory.

The loss of electrons on the absorption of ultraviolet light should also affect the action of colloids toward electrolytes. The precipitation of colloids by electrolytes is supposed to be due to neutralization or reversal of the electric charge on the colloid particle by the opposite charge on the precipitating ion. In the particular case of precipitation of egg albumin by ammonium sulphate we may picture the action of the electrolyte in the following way. As soon as the pH of a solution of egg albumin is made less than 4.8 some of the positively charged colloidal particles are precipitated by half saturation with ammonium sulphate. This is due to the bivalent anion SO_4'', which is known to be an effective precipitating agent toward positively charged colloids. The smaller the pH of the solution, the greater the positive charge on the particles of egg albumin and the greater the precipitation with half saturation, which becomes practically complete at pH 3.0. The experiments were not carried below pH 3.0. For pH greater than 4.8 there is no precipitation at half saturation but it occurs when a greater amount of ammonium sulphate is added. Now Sorensen (10) has shown that in a solution of egg albumin, containing ammonium sulphate, the albumin combines with the ammonia ion on the alkaline side of the isoelectric point. The amount of $(NH_4)^+$ combined with the negatively charged albumin particles increases with increasing pH and also with the concentration of ammonium sulphate. It therefore seems probable that this combination with $(NH_4)^+$ reverses the sign on the colloidal particles when the concentration of ammonium sulphate is great enough. They then become positive and can be precipitated by the SO_4'' ion, so that, although half saturation with ammonium sulphate does not precipitate negatively charged egg albumin, a greater concentration will do so.

After radiation, the solution with pH 4.0, having a greater positive charge should give a larger precipitate with half saturation than before radiation, and solutions with pH 4.8, 5.6, 6.2, 6.8, 7.2 and 7.6, which give no precipitate with half saturation before radiation, might be expected to give at least a partial precipitation after radiation.

These results, predicted theoretically on the assumption that the action of ultraviolet light on egg albumin is photo-electric, are entirely confirmed by the results given below.

METHOD. The white of a fresh egg was diluted to twice its volume with distilled water. The globulins were precipitated by half saturation with ammonium sulphate and removed by centrifuging and filtering. The albumin solution was then dialysed for two days against distilled water which was frequently changed. The solution thus obtained was found to have a pH of 6.2. By means of indicators, solutions were prepared with pH ranging from 4.0 to 8.2. They were exposed to the quartz mercury arc, in quartz and glass test tubes, for a succession of fifteen-minute periods, at a distance of five inches. The temperature of the solutions did not rise above 32°C. during the exposure.

RESULTS: I. *Effect of radiation through glass.* No effect was found at any pH when a solution was exposed to the quartz mercury arc in a glass test tube.

II. *Effect of radiation through quartz.* If solutions with pH 4.0, 4.8, 5.6, 6.2, 6.8, 7.2, 7.6, 8.2 are radiated in quartz test tubes the following results are obtained. Solutions with pH 4.0, 4.8, 7.2, 7.6, 8.2 remain clear. Those with pH 5.6, 6.2, 6.8 become cloudy after an exposure of about twenty minutes. This cloudiness increases with length of radiation although in 6.8 it never becomes very great. In 6.2 the solution becomes very thick and milky but no precipitate forms and it can not be filtered clear. On half saturation with ammonium sulphate both 6.2 and 6.8 precipitate leaving a clear filtrate, which after long enough radiation is free from albumin. Before radiation no precipitate was formed with half saturation. In 5.6 a precipitate settles out spontaneously on radiating and the filtrate is clear, no ammonium sulphate being necessary to remove the albumin. In pH 4.0 more albumin is precipitated with half saturation after radiation than before. Solutions with 4.8, 7.2, 7.6 and 8.2, in spite of being clear after radiation, precipitate on half saturation with ammonium sulphate, whereas before radiation no precipitate was formed. In 4.8 a short radiation converts all the uncharged to positively charged albumin which is completely precipitated by half saturation. In 7.2, 7.6 and 8.2 a very long radiation is necessary to complete the change, the particles having a large negative charge to begin with. The fact that solutions with pH greater than 6.8 do not show opalescence after radiation is probably due to the fact that, at that distance from the isoelectric point, the initial dispersion of the solution is so great that visible aggregation does not take place even when some of the particles become positively charged. A 5.4 solution precipitates in fine particles, 5.8 in large loose flakes, and 6.2, though opalescent, does not precipitate at all, showing that, although radiation gives a greater aggregation in all solutions with pH greater than 4.8, the degree of aggregation becomes steadily less with increasing alkalinity. A series of radiated tubes is shown in figure 1 and the results are tabulated in table 1.

The fact that it takes longer to complete the reaction in 6.2 than in 7.2 is probably due to the heavy opalescence in 6.2 which decreases the penetration of the light.

III. *The nature of the precipitate.* Schanz (3) has published work showing that, after radiation with ultraviolet light, egg albumin is more easily precipitated by half saturation with ammonium sulphate. He took this to mean that the albumin had been changed to globulin. This, however, is the wrong interpretation. The precipitate that settles out on radiating a pH 5.6 solution is not globulin but coagulated albumin for it does not dissolve in 1 per cent sodium chloride or in concentrated hydrochloric acid. The albumin is changed by radiation and becomes positively charged albumin, after which it is precipitated like globulin by half saturation with ammonium sulphate.

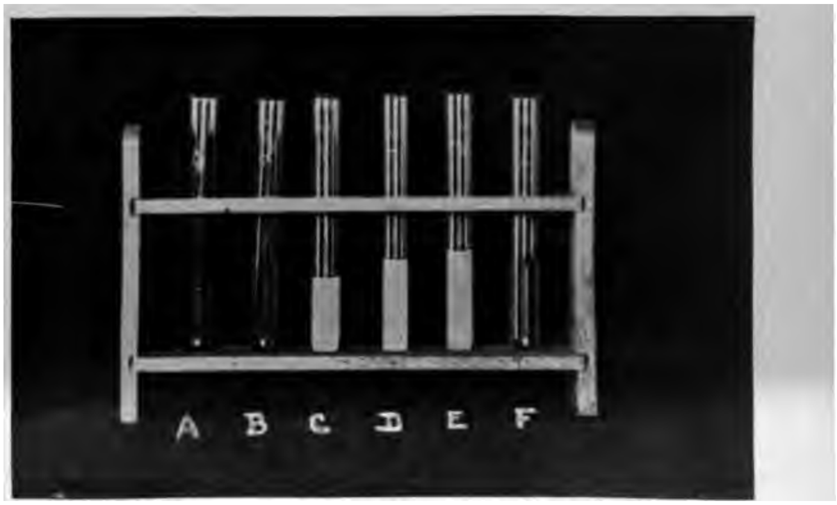

Fig. 1. A series of tubes containing egg albumin radiated one hour in quartz tubes. Tube *A* has pH = 3.8; tube *B*, 4.8; tube *C*, 5.6; tube *D*, 6.2; tube *E*, 6.8; tube *F*, 7.8. The difference in the opacity of *C*, *D* and *E*, which was in reality very marked, does not show in the plate.

IV. *Change in hydrogen ion concentration of radiated solutions.* Since the hydrogen ion concentration of a solution has an effect on the nature and amount of the electric charge on the colloidal particles, it is possible that the discharge of electrons from the colloidal particles might influence the hydrogen ion concentration of a solution. Observations were impossible, by the colorimetric method used, in the region pH 4.8 to 6.8 where the solutions became opalescent on radiation. Solutions with pH 3.6 and 7.2 were therefore radiated in quartz and glass test tubes and compared every hour with unradiated controls. In neither case was the solution radiated through glass affected even after a four-hour exposure. The pH 3.6 solution remained unchanged after three hours' radiation through quartz but showed a slight change in the direction pH 3.4 after four hours. The pH 7.2 solution however began to show a change at the end of an hour and after four hours radiation through quartz it had a pH of 6.8. The change was permanent since a radiated solution left over night, before the indicator was added, also had a pH of 6.8.

TABLE 1

Effect of ultraviolet radiation on egg albumin (through quartz)

pH	CHARACTER OF SOLUTION AFTER RADIATION	EFFECT OF ONE-HALF SATURATION WITH $(NH_4)_2SO_4$ BEFORE RADIATION	EFFECT OF ONE-HALF SATURATION WITH $(NH_4)_2SO_4$ AFTER RADIATION	TIME OF RADIATION NECESSARY TO COMPLETE THE PRECIPITATION BY ONE-HALF SATURATION
				hours
4.0	Clear	Small precipitate	Heavy precipitate	1¼
4.8	Clear	No precipitate	Heavy precipitate	1¼
5.6	Precipitate in heavy flakes. Clear on filtering	No precipitate	Heavy precipitate	1½
6.2	Thick opalescence on filtering	No precipitate	Heavy precipitate	3¾
7.2	Clear	No precipitate	Heavy precipitate	3
7.6	Clear	No precipitate	Heavy precipitate	4
8.2	Clear	No precipitate	Heavy precipitate	Not complete in 4 hours

V. *The effect of sensitizers.* In the presence of dyes the action of light on egg ablumin through quartz may be delayed or hurried, and in the presence of some the same reaction takes place through glass that ordinarily takes place only though quartz. The pH of the egg albumin solution was 6.2 before the dyes were added. In adding the dyes, 0.1 cc. of a 0.1 N aqueous solution was added to 1.0 cc. of the solution of egg albumin (table 2).

When eosin, erythrosin or rose bengal is added to a solution of egg albumin at pH = 4.8 a precipitate is formed on radiation which is not formed in the unsensitized solution at this pH. This may be due to a shift toward greater pH on the addition of the sensitizer, or else the isoelectric point of egg albumin may be different in the presence of a dye. Either explanation is likely and this point will be tested by means of electrometric hydrogen ion determinations.

TABLE 2

Sensitizers

SUBSTANCE	PRECIPITATED BY ONE-HALF SATURATION	PRECIPITATED BY RADIATION AND ONE-HALF SATURATION		TIME OF RADIATION NECESSARY FOR COMPLETE PRECIPITATION WITH ONE-HALF SATURATION	
		Quartz	Glass	Quartz	Glass
				hours	*hours*
Albumin + eosin	−	+	+	1½	4
Albumin + erythrosin	−	+	+	1⅔	3⅓
Albumin + rose bengal	−	+	+	2⅓	Not complete in 2½ hours
Albumin + methylene blue	−	+	+	2⅓	Not complete in 2½ hours
Albumin + uranin	−	+	+	Not complete in 2⅔ hours	Not complete in 2⅔ hours

Non-sensitizers

				hrs.	
Albumin + fuchsin	−	+	−	2⅔	
Albumin + methyl orange	−	+	−	3	

Since the coagulation of egg albumin by ultraviolet light through quartz is explained so perfectly on the assumption that it is due to photo-electric action, it seems more than probable that the coagulation through glass, in the presence of a sensitizer, is due to the same cause. This would mean that the photo-electric threshold of egg albumin is shifted to longer wavelengths in the presence of a sensitizer.

CONCLUSIONS

It has been shown that, due to the photo-electric action of ultraviolet light on egg albumin, radiation produces a state of greater aggregation when the albumin particles are negatively charged and a state of greater dispersion when they are uncharged or charged positively.

Although other colloidal solutions have not been worked with as yet, the principle is undoubtedly of general application and should explain, in a simple and satisfactory way, many of the physiological actions of light. So far no attention has been paid to the importance of the nature of the electrical charge on a substance that is radiated by ultraviolet light. A study of the effect of light on body tissues from this point of view promises most interesting results.

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THE NATURE OF SECONDARY VALENCE

BY HOMER W. SMITH

PART III—PARTITION COEFFICIENTS IN THE SYSTEM WATER: ETHER

§ 1. Introduction

In the preceding paper a method of correction was given which was designed to eliminate the disturbing influences of dissociation, association, etc., from partition coefficients. This method of correction consisted of plotting the ratio C_2/C_1 against C_2 (where C_2 and C_1 are the concentrations of the solute in the organic solvent and the water layers, respectively), and interpolating the value of C_2/C_1 when C_2 has a given unitary value, such as 1 millimol per litre. The value of the ratio C_2/C_1 so obtained was called the partition coefficient, $\mathfrak{P}$. The partition coefficient of a large number of organic acids and bases were determined for the systems water: xylene and water: chloroform.

It was shown that if the logarithms of the partition coefficients, $\mathfrak{P}$, for various molecular species were plotted against the irrespective volumes, the observed points fell into several straight and parallel lines, these lines being separated from each other along the ordinates by arithmetically equal values of $\mathfrak{P}$. The compounds which fell upon a given line were arbitrarily called a "series."

Ether was chosen for further work in partition coefficients largely because of its solubility in water. At 25°, water dissolves about 6 percent by weight of ether, and ether dissolves about one percent of water. This is nearly ten times the mutual solubility of water and chloroform. Thus the use of ether makes it possible to observe the extent to which the mutal solubility of the solvents affects the comparative results. Furthermore, ether is a good extractive of the organic acids and it is possible to study many compounds having very low partition coefficients which could not be studied in the systems previously used.

The ether was carefully repurified by distilling over calcium chloride and calcium oxide. Equal volumes of ether and of the aqueous solutions of known titer were measured into graduated flasks, and then mixed and shaken in separatory funnels after having been brought to the correct temperature. Samples of the water phase were than withdrawn and titrated, and the concentration in the ether was determined by difference. The solutes were taken from the samples used in the previous systems without further attempts at purification.

2. General Considerations

The relations disclosed by the partition coefficients in the system water: ether are essentially like those previously discussed for the systems water: xylene and water: chloroform:

1. The observed points fall into several straight and parallel lines.
2. These lines (or series) are separated from each other by distances along the ordinates which are arithmetically equal (equal intervals of $\mathfrak{P}$) except that—
3. Since $\mathfrak{P}$ is a logarithmic function of molecular volume, the value of the interval separating any two corresponding series increases ten times with each increase in the absolute value of $\mathfrak{P}$.
4. The equation covering these straight line relationships has been previously given:

$$\text{Log } 100\,\mathfrak{P} = \frac{V_m}{60.00} \pm a$$

(V_m is the molecular volume and a has been arbitrarily called the "series constant.")

The data for the partition coefficients in the system water: ether have been given in the following tables. They are arranged by series (*i. e.*, those compounds falling in the same line) beginning with the highest observed series and passing regularly to the lowest. The logarithmic nature of this series progression results in the separation of the series by actual (or graphical) intervals which gradually diminish in a repetitive fashion, each group of eight series comprising an increase of ten times in the value of $\mathfrak{P}$ at a given volume. Consequently each series within one of these groups of eight[1] may be said to occupy a characteristic position, denoted by the "series number." This series number is derived directly from the series constant. As an illustration consider the first ten series given in Table II.

Series constant a	Antilog. of a	Series number
+1.41160	25.80	4.0x6.45
+1.28666	19.35	3.0x6.45
+1.11057	12.90	2.0x6.45
+0.80954	6.45	1.0x6.45
+0.71263	5.16	.8x6.45
+0.65464	4.515	.7x6.45
+0.58769	3.87	.6x6.45
+0.50851	3.225	.5x6.45
+0.41160	2.58	.4x6.45

The figures in the second column are related to each other as integral multiples of 6.45: hence the respective series may be most simply indicated by this multiple, or "series number," as given in column 3. These series numbers have been given a decimated form in order that their relative position may be identified more readily. That is, the series above the first series 1 have been numbered with integrals, the next group with tenths, the next with hundredths, and so on. This arrangement will be made clearer by reference to Figure 1.

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[1] The term "octave" was previously applied to each group of eight intervals, but it was based upon the erroneous idea that there were only eight possible series; this point is refuted in this paper and hence the term has been abandoned.

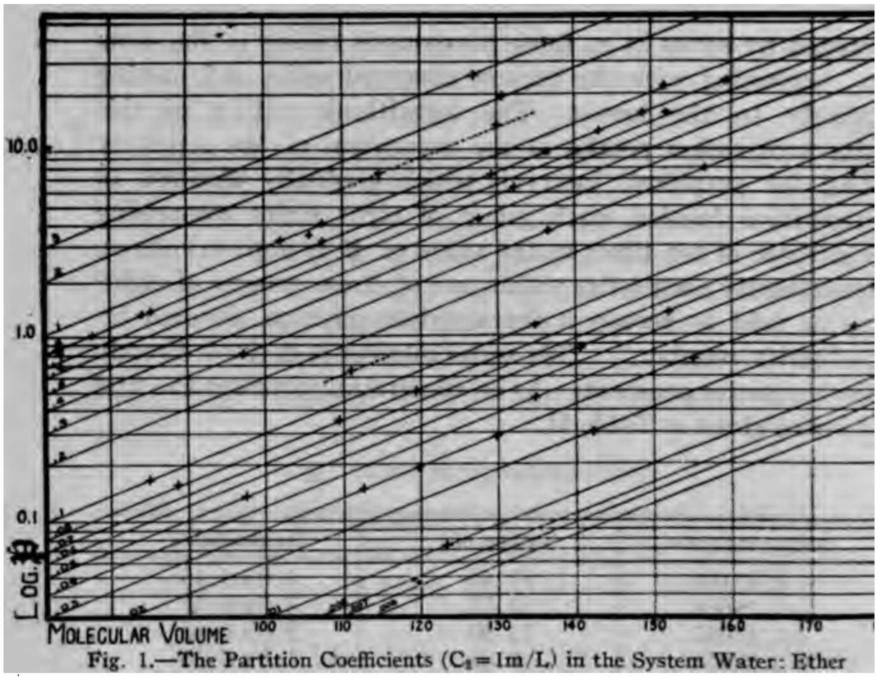

Fig. 1.—The Partition Coefficients (C_1 = 1m/L) in the System Water: Ether

TABLE I
Partition Coefficients of the Amines
Water: Ether

Series no.	a	V_m	C_2	β obs.	β calc.
.1	+0.39794				
	Methyl amine	43.0	1	0.113?	0.132
	Trimethyl amine	88.0	1	0.660?	0.732
.8	+0.30103				
.7	+0.24310				
	Dimethyl amine	66.3	1	0.231	0.224
.6	+0.17609				
	Ethyl amine	64.6	1	0.172	0.179
	n-Butyl amine	106.8	1	0.905	0.905
	iso-Amyl amine	127.0	1	2.02	1.96
	Benzyl amine	127.4	1	2.00	1.99
	Benzyl methyl amine	151.0	1	5.00	4.93
	n-Heptyl amine	169.5	1	10.3	10.03
	Benzyl ethyl amine	173.0	1	11.6	11.46
.5	+0.09691				
	Dipropyl amine	151.5	1	3.86	4.18
.4	±0.00000				
	Piperidine	108.8	1	0.655	0.651
	Diethyl amine	109.3	1	0.652	0.663
	n-Methylpiperidine	133.8	1	1.68	1.70

TABLE II
Partition Coefficients of the Acids
Water: Ether

Series no.	a	V_m	C_2	β obs.	β calc.
4.	+1.41160				
	Benzoic acid	126.9	10	33.0	33.6
3.	+1.28666				
	Benzoic acid		1	25.0	25.2
2.	+1.11057				
	Chloroacetic acid	75.0	100	2.5	2.58
	α-Bromopropionic acid	105.8	10	7.4	7.48
	β-Iodopropionic acid	115.0	10	10.0	10.65
	Salicylic acid	130.6	1	19.0	19.37
	Resorcinilic acid	136.3	10	23.0	24.11
1.	+0.80954				
	Formic acid	41.5	1	0.320	0.317
	α-Crotonic acid	102.1	1	3.20	3.24
	α-Bromopropionic acid	105.8	1	3.48	3.74
	n-Butyric acid	107.8	1	4.05	4.04
	p-Oxybenzoic acid	132.3	10	10.2	10.34
	o-Nitrobenzoic acid	150.8	1	2.17	2.103
.8	+0.71263				
	Thiacetic acid	73.6	1	0.84	0.869
	Chloroacetic acid	78.0	1	1.02	1.03
	Bromoacetic acid	84.5	1	1.32	1.37
	Propionic acid	85.3	1	1.32	1.36
	sec-Butyric acid	107.3	1	3.22	3.17
	iso-Valeric acid	129.5	1	7.40	7.43
	Resorcinilic acid	136.3	1	9.40	9.64
	Gentisinic acid	136.5	10	10.3	9.72
	o-Chlorobenzoic acid	143.3	1	12.5	12.60
	Phenylacetic acid	148.9	1	15.2	15.60
	o-Iodobenzoic acid	159.4	1	23.0	23.40
.7	+0.65464				
	Acetic acid	64.0	1000	0.535	0.526
	Dichloroacetic acid	97.2	10	1.9	1.882
	m-Nitrobenzoic acid	151.9	1	15.5	15.4
.6	+0.58769				
	Acetic acid	64.0	1	0.450	0.451
	p-Oxybenzoic acid	132.3	1	6.2	6.20
.5	+0.50851				
	α-β-Dibromopropionic acid	127.8	1	4.20	4.35

TABLE II (*Continued*)

Series no.	a	V_m	C_2	𝔓 obs.	𝔓 calc.
.4	+0.41160				
	3.5-Dinitrobenzoic acid	175.8	10	21.0	21.96
.3	+0.28666				
	Dichloroacetic acid	97.2	1	0.805	0.8066
	Gentisinic acid	136.5	1	3.6	3.64
.2	+0.11057				
.1	−0.19046				
	Pyruvic acid	85.3	1	0.162	0.170
	Anthranilic acid	135.0	1	1.11	1.147
	Oxalic acid	75.6	10	0.116	0.119
	Lactic acid	88.7	10	0.191	0.194
	Bromosuccinic acid	140.5	10	1.38	1.417
.08	−0.28737				
	Oxalic acid	75.6	1	0.092	0.094
	Lactic acid	88.7	1	0.155	0.155
	β-Oxybutyric acid	109.7	1	0.353	0.348
	Trichloroacetic acid	119.5	1	0.495	0.506
	Mandelic acid	152.2	10	1.66	1.775
	Acetylsalicylic acid	185.6	10	6.7	6.397
.07	−0.34536				
	2.4-Dinitrobenzoic acid	175.3	10	3.6	3.77
.06	−0.41231				
	Bromosuccinic acid	140.5	1	0.84	0.85
	Mandelic acid	152.2	1	1.30	1.33
	Acetylsalicylic acid	185.6	1	4.75	4.80
.05	−0.49149				
	Malonic acid	97.8	1	0.134	0.1376
	Benzilic acid	238.3	10	31.8	30.21
.04	−0.58840				
	Gallic acid	135.0	1	0.451	0.4588
.03	o-Phthalic acid	155.6	10	1.04	1.072
	−0.71334				
	Maleic acid	112.6	1	0.150	0.1457
	Succinic acid	120.0	1	0.192	0.193
	Laevulinic acid	130.0	1	0.280	0.284
	o-Phthalic acid	155.6	1	0.740	0.758
.02	Benzilic acid	238.3	1	18.0	18.1
	−0.88943				
	Glutaric acid	142.2	1	0.296	0.302
.01	2.4-Dinitrobenzoic acid	175.3	1	1.10	1.08
	−1.19046				
.008	Tartaric acid	123.6	1	0.072	0.0740
.007	−1.28737				
	−1.34536				
.006	2.4.6-Trinitrobenzoic acid	197.0	10	0.88	0.867
	−1.41231				
.003	Hippuric acid	178.7	1	0.379	0.368
	−1.71334				
	2.4.6-Trinitrobenzoic acid	197.0	1	0.36	0.371

TABLE III

The Evaluation of Series Which Are Not Integrally Related

	V_m	C_2	[illegible] obs.	*a* calc.	Series no. corresponding to *a*
Ether					
Formic acid	41.5	10	0.430	+0.94181	
Bromoacetic acid	84.5	10	2.27	+0.94770	
n-Butyric acid	107.8	10	5.20	?(+0.91767)	
β-Iodopropionic acid	115.0	1	7.3	+0.94666	
n-Valeric acid	130.0	1	13.54	+0.96464	
m-Nitrobenzoic acid	151.9	10	31.00	+0.95970	
			Mean	+0.95210	1.388
α-Oxybutyric acid	111.2	1	0.633	—0.05193	
3.5-Dinitrobenzoic acid	175.8	1	7.7	—0.04351	
			Mean	—0.04772	.1389
Chloroform					
β-Oxybutyric acid	109.7	1	0.196	—0.53607	.1353
Xylene					
n-Caproic acid	152.5	1	2.37	—0.16691	1.588
			Mean for Series no.		1.406
Ether					
Chloroacetic acid	78.0	10	1.58	+0.89866	1.227
Fumaric acid	112.6	1	0.58	—0.07556	.1190
Xylene					
iso-Caproic acid	152.0	1	1.75	—0.29029	1.192
			Mean for Series no.		1.203
Ether					
Trichloroacetic acid	119.5	10	1.50	+0.18443	.237

There are ten instances which clearly indicate the existence of a series midway between Series 1 and 2. The exact value of this series has arbitrarily been placed at 1.414, or the square root of two (obs. 1.406). There are three instances indicating the existence of another series between this and Series 1, which have the average value 1.203. The remaining compound, trichloroacetic acid, falls between Series .2 and .3 (obs. .237). The last two cases will be disregarded for the time being, but the evidence is sufficient to establish the existence of a series between Series 1 and 2, and in subsequent discussions this will be recognized as Series 1.414.

3. The Interpolation Value of C_2

In the previous communication only those partition coefficients were considered which were derived by interpolating to one millimol per litre in the organic solvent. Though a detailed discussion of the method of correction cannot be attempted at this time, it may be pointed out that this method is of such a nature that the general interpretation should be no different were it based upon results obtained from interpolations to any other series of geometrically related values, such as 10, 100 or 1000 millimols per litre. (For that matter, one might choose any geometrically related series of numbers such as 2, 20, 200, etc., 3, 30, etc., for the interpolation concentrations.) Practical difficulties prevent the determination of distribution ratios for many substances at such concentrations that we may interpolate to 1000 or even 100 m/L, but in the majority of instances it is possible to make this interpolation to 10 m/L. At the time this experimental work was done it was not intended to make such an interpolation, and consequently it is possible to do so on only a part of the available data. These values, derived from 10 m/L, are included in the preceding tables. The figures in the third column give the value of C_2 to which interpolation has been made.

4. The Inter-relations of Series

On the basis of the results obtained in the systems water: xylene and water: chloroform it was stated that the observed values of $\mathfrak{P}$, when plotted against molecular volume, fell into parallel lines or series which are separated from each other by distances (in terms of $\mathfrak{P}$) which *are related as the integrals from one to 10.* (Series 9 apparently is missing.) This statement, though well supported by the evidence under consideration, must be modified in view of the evidence furnished by the system water: ether. This latter evidence shows that there are series which are not taken into account by this simple integral relationship. The data bearing upon these additional series are given in the following table.

The series constants *a* which are given in the fourth column have been calculated from the observed partition coefficients. The series number is obtained from these series constants by subtracting the value of *a* for Series 1, and converting the difference to the antilog. Some additional data have been included from the systems water: xylene and water: chloroform.

5. The Blank Series

In ether, as in xylene and chloroform, some series appear to be more favored than others, so far as the number of observed cases occurring in them is concerned. Thus the number of compounds occurring in the various series is as follows:

Series numbers				Acids $C_2 = 1$ m/L	$C_2 = 10$ m/L	Amines $C_2 = 1$ m/L
1	.1	.01		8	4	2
2	.2	.02		2	4	0
3	.3	.03		9	4	0
	.4	.04		1	3	3
	.5	.05		2	1	1
	.6	.06	.006	6	0	7
	.7	.07		1	4	1
	.8	.08		14	3	0

Considerable emphasis was previously laid on the fact that some of these series, though theoretically called into existence by their simple inter-relationships, were apparently blank, in the sense that no compounds were observed in them. The existence of these so-called "blank series" is not borne out by this study. Thus among the acids at $C_2 = 1$ m/L, Series 2, 4, 5 and 7 contain the smallest number of compounds, whereas among the amines, the minima are Series 2, 3, 5, 7 and 8. In xylene and chloroform, Series 5, 7 and 8 were blank with both the amines and the acids. Thus there is a tendency for the amines to congregate in the same series in xylene, chloroform and ether; but with the acids, if any series at all may be considered as favored in ether, they are certainly not the same as those favored in xylene and chloroform. It is evident that we cannot describe any series as being definitely blank, but that the appearance and identity of such series depends upon the particular solvents under consideration. However, the point that some series may be differentiated from other series under particular conditions, cannot be held to be without significance in view of the relations previously discussed in this connection[1] though the reason for this differentiation is not evident at the present time.

6. The Absolute Values of the Series Constants

The series constants in the system water:ether (a_e) are related to the series constants in the system water:xylene (a_x) by a constant difference:

$$\text{(Acids) } a_e = a_x + \log 15.0$$
$$\text{(Amines) } a_e = a_x + \log 1.50$$

Thus each series in the system water:ether has a value in terms of $\mathfrak{P}$, at a given molecular volume, just 15.0 or 1.5 times its value at that same volume in the system water:xylene.[2]

The exact value of any series constant in this, as in other cases, is theoretically determined by extrapolating from the observed points to $V_m = 0$ according to Equation I. But it could not be expected that such a method would yield outright a pure integral relationship such as 15.00; it must be admitted that this evaluation is based upon the assumption that the series constants for various solvents are also integrally related. It may be reiterated here that a similar integral relation existed between the systems water:xylene and water: chloroform;

$$\text{(Acids) } a_c = a_x + \log 5.00$$
$$\text{(Amines) } a_c = a_x + \log 5.00$$

Other systems containing water will be considered later, when it will be seen that this integral relationship is just as true for various solvents as for the solutes.

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[1] Jour. Phys. Chem., 25, 160, 204 (1921).

[2] The system water: ether is the only one in which the acids and amines behave differently in this respect. It is noteworthy that here the difference is exactly 10 to 1.

Summary

The partition coefficients of 49 organic acids and 15 organic bases have been determined in the system water: ether at 25°. The results obtained from a comparative treatment of these partition coefficients in terms of molecular volume are in good agreement with the results obtained in the systems water:xylene and water:chloroform. For a detailed discussion on these results, the reader is referred to the previous communication.[1]

It is realized from the consideration of further evidence that the interpretation of these results may have to be modified in some respects, but no attempt will be made in this direction until all the experimental evidence has been presented.

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[1] Jour. Phys. Chem., **25**, 160, 204 (1921).

School of Hygiene and Public Health
Department of Physiology
Baltimore, Maryland

THE NATURE OF SECONDARY VALENCE

BY HOMER W. SMITH

Supplementary Note on the Method of Correction

The recognition of series other than the eight which have been hitherto considered raises a question as to the fundamental nature of this rhythmic behavior and the validity of the interpretation based upon it. No entirely satisfactory answer can be made at the present time, but a careful consideration of the evidence discloses something of the nature of the phenomena with which we are dealing.

In the first place it can be seen from the data which have been given that in passing from concentrated to dilute solutions, the partition coefficient moves progressively through one or more series. A few examples are given below.

	C_2	Series number	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
ETHER				
Dichloroacetic acid	10	.7	1.9	1.88
	1	.3	0.805	0.806
Oxalic acid	10	.1	0.116	0.119
	1	.08	0.092	0.094
Chloroacetic acid	1000	2.	2.5	2.58
	1	.8	1.02	1.03
CHLOROFORM				
Acetic acid	1000	1.0	0.25	0.25
	100	.4	0.10	0.1002
	10	.1732 (1)	0.438	0.4322
	1	.1	0.025	0.025
Chloroacetic acid	1000	.3	0.125	0.1287
	100	.1414 (2)	0.062	0.0620
	10	.08	0.0342	0.0343
	1	.04	0.0168	0.01716

(1) $0.1732 = \sqrt{3}$
(2) $0.1414 = \sqrt{2}$

In the second place, the distribution ratios for many substances are essentially constant during dilution. (Cf. acetic, anthranilic, sec-butyric acids, etc., in the following experimental section.) In the third place, there are instances in xylene and chloroform where the distribution ratio increases with dilution. (Cf. *iso*-amylacetic acid ethylamine and methylamine, etc.)

Nevertheless, by interpolating at geometrically related points in these processes, we obtain values which are consistently related in a regular manner. In these processes, we are dealing with a stoichiometric complex (association, dissociation, hydration, etc.), which "decomposes" at a given rate as we pass from concentrated to dilute solutions. If we look upon this decomposition, expressed in the ratio C_2/C_1, as exponential in character, then by means of the geometrically related interpolations which we are making we are cross-sectioning the "behaivor," so to speak, of various molecular species under comparable conditions. The fact that the relation C_2/C_1 is never of a type which can be covered throughout all dilutions by a single exponential equation has been repeatedly demonstrated. Where one exponent covers it through a given range, another exponent must be used to cover it through an adjacent range. In order to cover the entire range from very concentrated to very dilute solutions, it would be necessary to assume transitions from one exponent to another;

$$C_2/Cx_1 = a; \quad C_2/Cy_1 = b; \quad C_2/C_{z1} = c; \text{ etc.}$$

The fact that this interpolation treatment reveals such simple arithmetic relations in relative solubility and stoichiometric behavior would indicate that similar arithmetical relations exist in those forces which give rise to the stoichiometric complex. (In view of the existence of non-integral series such as 1.414, etc., it is perhaps unwise to use the term *integral* in this connection; but the same idea is conveyed and the facts more accurately described, by the term simple, *arithmetical* relations.)

As a matter of fact, the interpolation treatment *could not* yield intelligible results were the initial forces in the stoichiometric complex continuous, instead of discontinuous, variables; nor, if these forces were continuous variables, could it be directly demonstrated that molecular volume played any rôle in intermolecular relations, for if there is to be *more than one series*, the various series must be separated by at least *definite*, if not *related*, amounts.

And in view of our present notions of the structure of matter, it is not difficult to believe that the various degrees in discontinuity in the fundamental forces are related in some simple manner.

SECTION III

Experimental[1]

Note of Correction.—The data for the partition of acetic and chloroacetic acids between water and chloroform which were given in Section II (pp. 227, 228) of this paper are incorrect. The figures given represent the distribution between one volume of water and three volumes of chloroform. Correct figures for acetic acid were given in Section I (p. 215), $\mathfrak{P}$ (1000 M/L) = 0.25; $\mathfrak{P}$ (100 M/L) = 0.10. The data for chloroacetic acid are as follows:

	C_1	C_2	C_2/C_1	$\mathfrak{P}$
Chloroacetic acid (Chloroform)	194.25	5.50	0.0283	*10*
	199.00	5.50	0.0276	0.034
	86.50	1.6875	0.0195	
	68.25	1.250	0.0183	*1*
	42.75	0.625	0.0146	0.0168

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[1] C_1 = concentration in water; C_2 = concentration in organic solvent. The italicized figures in the last column indicate the concentration in the organic solvent (C_2) to which interpolation is made to obtain $\mathfrak{P}$. Thus for chloroacetic acid, when C_2 = 10 M/L, $\mathfrak{P}$ = 0.034; and when C_2 = 1 M/L, $\mathfrak{P}$ = 0.0168.

The Partition Coefficients of the Acid between Water and Ether, 25°

	C_1	C_2	C_2/C_1	$\mathfrak{P}$
Acetic acid[1]	1919.0	1026.0	0.535	
	1156.0	616.0	0.532	
	333.0	168.0	0.505	Mean
	73.3	416.0	0.568	0.535
Acetic acid	14.4	6.75	0.468	
	8.90	4.20	0.472	
	5.20	2.35	0.452	
	2.8625	1.2875	0.449	Mean
	1.075	0.475	0.442	0.456
Acetyl salicylic acid	1.32	8.84	6.7	*10*
	0.84	5.16	6.15	6.7
	0.49	2.75	5.62	
	0.31	1.57	5.06	*1*
	0.205	0.96	4.70	4.75
Anthranilic acid	6.04	6.80	1.125	
	3.57	4.04	1.130	
	1.86	2.21	1.190	
	0.100	1.05	1.050	Mean
	0.520	0.560	1.075	1.11
Benzilic acid	0.300	9.22	30.7	*10*
	0.160	4.44	27.7	31.8
	0.1200	2.72	22.65	
	0.0775	1.55	20.00	*1*
	0.0500	0.83	16.60	18.00
Benzoic acid	0.370	13.75	37.80	*10*
	0.270	8.130	30.15	33.0
	0.1575	4.8625	30.85	
	0.095	2.3850	25.15	
	0.065	1.6250	25.00	*1*
	0.0375	0.9125	24.30	25.00
Bromoacetic acid	4.25	9.55	2.245	*10*
	3.05	6.30	2.063	2.27
	1.475	3.475	1.760	
	1.3125	1.8875	1.438	*1*
	0.9625	1.3375	1.390	1.32

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[1] Morgan and Benzon: Zeit. anorg. Chem., 55, 356 (1907).

	C_1	C_2	C_2/C_1	B
α-Bromopropionic acid	1.725	2.275	7.11	*Io*
	1.30	9.50	7.30	7.4
	1.125	6.675	5.93	
	0.8625	4.8625	5.64	
	0.700	3.450	4.93	
	0.5875	2.7875	4.75	
	0.500	2.275	4.55	
	0.40875	1.68125	4.02	*I*
	0.3125	1.1250	3.60	3.48
Bromosuccinic acid	6.70	8.90	1.35	*Io*
	3.50	3.85	1.10	1.38
	2.00	1.925	0.963	
	1.3625	1.1625	0.853	*I*
	0.63125	0.4500	0.712	0.84
n-Butyric acid	2.64	14.04	5.32	*Io*
	1.80	9.20	5.12	5.2
	1.60	5.02	4.73	
	0.59	2.65	4.50	*I*
	0.335	1.40	4.18	4.05
s-Butyric acid	2.6	8.4	3.23	
	1.625	5.575	3.43	
	0.975	3.225	3.30	
	0.500	1.6125	3.22	Mean
	0.29375	0.85625	2.92	3.22
Chloroacetic acid	8.58	15.20	1.770	*Io*
	7.80	13.40	1.718	1.58
	5.11	8.48	1.66	
	4.60	6.70	1.455	*I*
	2.85	3.70	1.299	1.020
	1.9375	2.3375	1.208	
	1.40	1.55	1.08	
o-Chlorobenzoic acid	1.360	9.68	26.80	*Io*
	0.250	5.67	22.65	27.1
	0.1925	3.7075	19.30	
	0.145	2.415	16.65	*I*
	1.100	1.300	13.00	12.5
α-Crotonic acid	3.52	11.6	3.30	
	1.68	5.48	3.26	
	1.24	3.92	3.16	
	0.85	2.73	3.21	
	0.54	1.70	3.15	

	C_1	C_2	C_2/C_1	[illegible]
	0.52	1.60	3.08	Mean
	0.32	0.95	2.96	3.20
α-β-Dibromopropionic acid	0.450	3.2625	7.25	
	0.350	2.1000	6.00	
	0.25625	1.13125	4.42	*1*
	0.21875	0.85625	3.92	4.20
Dichloroacetic acid	9.5	25.9	2.72	*10*
	6.5	14.8	2.28	1.9
	4.05	6.95	1.72	
	2.325	2.675	1.15	*1*
	1.25	1.025	0.82	0.805
2.4-Dinitrobenzoic acid	2.495	7.78	3.12	*10*
	1.79	4.08	2.28	3.6
	1.45	2.51	1.73	
	1.15	1.69	1.41	*1*
	0.67	0.68	0.91	1.10
3.5-Dinitrobenzoic acid	0.365	6.395	17.5	*10*
	0.295	3.885	13.2	21.10
	0.225	2.655	11.8	
	0.1850	1.815	9.8	*1*
	0.1325	1.0475	7.9	7.7
Formic acid	30.00	13.25	0.442	*10*
	13.45	4.75	0.353	0.43
	8.85	3.25	0.368	
	6.55	2.30	0.351	
	4.775	1.625	0.340	0.320
Fumaric acid	8.85	6.75	0.763	
	4.80	3.50	0.729	
	2.60	1.70	0.654	
	1.5125	0.8375	0.554	*1*
	1.01875	0.4625	0.454	0.58
Gallic acid	59.5	27.0	0.453	
	35.2	16.10	0.457	
	22.0	10.00	0.454	
	15.75	7.13	0.452	
	8.25	3.72	0.452	Mean
	2.82	1.27	0.451	0.451
Gentisinic acid	1.12	11.92	10.65	*10*
	0.83	7.65	9.22	10.3
	0.60	3.98	6.64	
	0.41	2.07	5.05	*1*

	C_1	C_2	C_2/C_1	𝔓
	0.31	1.205	3.89	3.6
Glutaric acid	11.90	3.65	0.306	
	6.40	1.875	0.292	
	3.0875	0.9125	0.296	
	1.300	0.34375	0.264	*1*
	0.70625	0.18750	0.268	0.296
Hippuric acid	8.04	2.80	0.348	
	4.66	1.70	0.364	
	2.58	0.98	0.380	
	1.42	0.56	0.394	*1*
	0.855	0.355	0.415	0.376
o-Iodobenzoic acid	0.1010	2.640	26.2	
	0.0602	1.400	23.2	
	0.0400	0.861	21.5	*1*
	0.0238	0.505	21.2	23.0
β-Iodopropionic acid	1.44	15.22	10.6	*10*
	0.900	8.66	9.6	10.0
	0.500	4.64	9.3	
	0.280	2.26	8.1	*1*
	0.175	1.275	7.3	7.3
Lactic acid	28.3	5.10	0.180	*10*
	15.7	2.70	0.172	0.191
	9.35	1.525	0.163	
	5.95	1.000	0.155	*1*
	3.95	0.550	0.139	0.155
Laevulinic acid	14.65	3.75	0.2575	
	10.10	2.65	0.2625	
	5.15	1.40	0.2725	
	2.57	0.725	0.2820	*1*
	1.725	0.500	0.2900	0.280
Maleic acid	11.45	1.90	0.166	
	7.25	1.025	0.141	
	4.85	0.75	0.153	
	2.50	0.35	0.140	*1*
	1.56	0.215	0.138	0.150
Malonic acid	94.60	14.0	0.148	
	34.42	5.17	0.150	
	22.30	3.44	0.154	
	7.90	1.05	0.133	
	3.80	0.5125	0.135	*1*
	2.1875	0.29375	0.134	0.134

	C_1	C_2	C_2/C_1	$\mathfrak{p}$
Mandelic acid	5.225	8.525	1.63	*10*
	3.225	4.825	1.495	1.66
	2.100	2.95	1.405	
	1.070	1.475	1.380	*1*
	0.885	1.175	1.330	1.30
m-Nitrobenzoic acid	0.240	6.812	28.8	*10*
	0.180	4.260	23.7	31.0
	0.135	2.905	21.5	
	0.095	1.875	19.7	*1*
	0.075	1.250	16.7	15.5
o-Nitrobenzoic acid	1.840	13.08	7.11	*10*
	1.200	6.96	5.80	7.1
	0.790	3.035	3.84	
	0.543	1.413	2.60	*1*
	0.379	0.663	1.75	2.17
Oxalic acid	84.25	9.60	0.113	*10*
	39.35	3.80	0.0965	0.116
	22.625	2.0375	0.0902	
	14.10	1.31	0.0930	*1*
	5.9375	0.525	0.0885	0.092
p-Oxybenzoic acid	1.4	12.8	9.15	*10*
	0.875	7.425	8.50	10.2
	0.575	5.025	8.72	
	0.400	3.250	8.13	*1*
	0.3125	2.2625	7.23	6.2
α-Oxybutyric acid	9.70	6.40	0.660	
	5.75	3.55	0.617	
	4.1375	2.7125	0.656	
	2.875	1.875	0.652	Mean
	1.7625	1.0875	0.617	0.633
β-Oxybutyric acid	6.60	2.36	0.356	
	4.75	1.70	0.358	
	3.05	1.075	0.352	
	1.725	0.600	0.348	Mean
	1.075	0.375	0.349	0.353
Phenylacetic acid	1.08	16.12	15.0	
	0.44	7.44	16.9	
	0.26	3.82	14.7	
	0.17	2.47	14.5	Mean
	0.085	1.255	14.7	15.2
Phthalic acid	6.675	6.575	0.985	*10*

	C_1	C_2	C_2/C_1	[illegible]
	3.800	3.375	0.888	1.04
	2.375	1.925	0.810	
	1.5375	1.100	0.716	*I*
	0.96875	0.66875	0.690	0.740
Propionic acid	5.45	8.30	1.52	
	2.35	3.375	1.435	
	0.925	1.225	1.325	*I*
	0.675	0.8875	1.315	1.32
Pyruvic acid	14.10	2.40	0.170	
	7.05	1.15	0.163	
	5.00	0.80	0.160	Mean
	3.65	0.55	0.151	0.162
Resorcinilic acid	0.50	12.75	25.50	*IO*
	0.3875	8.7125	22.50	23.0
	0.28125	4.51875	16.00	1
	0.1350	1.5950	11.81	9.4?
Salicylic acid	0.295	14.825	50.3	
	0.195	7.645	39.2	
	0.125	3.855	30.8	
	0.0850	2.415	28.4	*I*
	0.0666	1.3975	21.0	19.0
Succinic acid	13.8	2.75	0.199	
	7.95	1.55	0.195	
	4.3625	0.8625	0.1975	
	2.6250	0.4875	0.186	Mean
	1.6000	0.2880	0.180	0.192
Tartaric acid	98.75	6.0625	0.0613	
	37.56	2.5625	0.0683	
	21.62	1.50	0.0694	*I*
	8.40	0.625	0.0745	0.072
Thiacetic acid	9.70	8.5	0.877	
	6.00	5.3	0.884	
	3.175	2.675	0.843	
	1.39	1.165	0.839	*I*
	0.9675	0.8025	0.830	0.84
Trichloroacetic acid	8.825	17.00	1.928	*IO*
	6.640	10.62	1.600	1.50
	7.10	10.40	1.465	
	5.10	5.40	1.060	
	3.20	2.50	0.781	
	1.95	0.85	0.436	*I*

	C_1	C_2	C_2/C_1	[illegible]
	1.4625	0.5625	0.385	0.495
2.4.6-Trinitrobenzoic acid	8.28	5.88	0.710	*10*
	4.80	2.20	0.458	0.88
	2.56	0.84	0.328	*I*
	1.09	0.200	0.182	0.36
Valeric acid	0.3900	5.6700	14.52	
	0.3000	4.3000	14.32	
	0.1700	2.2000	12.95	
	0.1125	1.4575	12.92	**Mean**
	0.0775	1.0225	13.20	**13.54**
iso-Valeric acid	1.325	11.0	8.32	
	0.8875	7.0875	7.98	
	0.5750	4.50	7.82	
	0.44375	3.275	7.70	*I*
	0.2500	1.875	7.50	7.04

**Partition Coefficients of the
Amines between Water and Ether, 25°**

Benzylamine	4.55	9.60	2.10	
	2.85	6.05	2.12	
	1.85	3.725	2.01	
	1.10	3.25	2.04	**Mean**
	0.675	1.335	1.97	**2.00**
Benzylethylamine	0.92	12.92	14.1	
	0.56	7.60	13.6	
	0.365	4.995	13.7	
	0.230	2.83	12.3	*I*
	0.145	1.74	12.0	11.6
Benzylmethylamine	2.08	12.84	6.17	
	1.14	5.66	5.84	
	0.645	3.665	5.69	
	0.41	2.12	5.17	*I*
	0.23	1.17	5.08	5.00
***n*-Butylamine**	3.20	3.52	1.10	
	2.15	2.20	1.02	
	1.35	1.23	0.912	*I*
	0.85	0.76	0.895	0.905
***iso*-Amylamine**	0.8375	1.8375	2.19	
	0.4875	1.0125	2.08	
	0.3750	0.6500	1.73	*I*
	0.2275	0.3875	1.63	2.02

	C_1	C_2	C_2/C_1	P
Coniine	1.06	7.38	6.95	
	0.62	3.72	7.16	
	0.33	1.875	5.68	
	0.19	1.055	5.55	6.33
Diethylamine	7.9	6.3	0.798	
	2.875	2.025	0.704	
	1.650	1.075	0.652	
	1.100	0.7375	0.671	*I*
	0.6125	0.4375	0.714	0.652
Dimethylamine	13.5	3.05	0.226	
	7.0	1.60	0.228	
	5.22	1.26	0.242	
	4.35	1.025	0.236	
	2.81	0.63	0.224	
	2.55	0.600	0.235	
	1.85	0.425	0.230	Mean
	1.37	0.33	0.241	0.231
Dipropylamine	1.48	9.76	6.6	
	0.92	5.70	6.2	
	0.60	2.97	4.95	
	0.43	1.915	4.46	*I*
	0.26	1.005	3.86	3.86
Ethylamine	14.8	2.50	0.169	
	9.8	1.60	0.163	
	6.075	1.125	0.185	
	3.975	0.675	0.170	Mean
	2.550	0.450	0.176	0.172
n-Heptylamine	0.385	8.00	20.8	
	0.275	5.145	18.7	
	0.200	3.105	15.5	*I*
	0.120	1.41	11.75	10.3
Methylamine	34.5	3.9	0.113	
	19.1	2.05	0.107	
	11.25	1.225	0.109	
	7.60	0.90	0.118	Mean
	4.90	0.575	0.117	0.113
n-Methylpiperidine	5.92	10.92	1.85	
	3.42	6.20	1.81	
	2.065	3.59	1.74	
	1.33	2.31	1.74	*I*
	0.805	1.37	1.70	1.68

	C_1	C_2	C_2/C_1	[illegible]
Piperidine	14.10	17.85	0.790	
	8.60	11.30	0.760	
	4.85	6.70	0.724	*I*
	2.60	3.80	0.685	0.655
	1.10	1.675	0.657	
Trimethylamine	6.30	8.90	0.707	
	3.00	4.45	0.675	
	1.775	2.65	0.670	
	1.1625	1.775	0.655	*I*
	0.800	1.2125	0.660	0.660

THE NATURE OF SECONDARY VALENCE. PART IV

BY HOMER W. SMITH

Partition Coefficients in the System Glycerine : Acetone

§ 1. Introduction

In the experimental work thus far presented, water has always been used as one of the solvents. Since water is unique in its physical properties, it seemed possible that the results obtained in these systems might be modified to a great extent in systems composed of non-aqueous liquids. For this reason, a large amount of work has been done in the system glycerine : acetone. Glycerine takes the part of water and acetone the part of the organic solvent.

In preliminary experiments, a commercial grade of glycerine was heated in a distilling flask at 140° C for two hours. This sample on trial gave the same results as did the original material and consequently the original was used with out preliminary heating.[1] It furthermore checked in a satisfactory manner other lots from the same and other sources so that it is improbable that any systematic error entered from this direction.

The acetone was carefully redistilled from a mixture of equal parts of calcium oxide and calcium chloride. Since the acid-acetone was repeatedly recovered in this manner, it is certain that the product was anhydrous and free from serious contaminations.

The acid phthalate salts were made by mixing standardized solutions of phthalic acid on the one hand, and the desired amine on the other, in the proportions of two to one. It was assumed that the remaining titratable hydrogen of the phthalic acid represented one molecule of the amine acid-salt.

It was necessary to design a special container for this study, since the glycerine cannot be measured from one flask to another with any accuracy. A number of glass, pistol-shaped flasks were devised. Each consisted of a bulb of definite volume (corresponding to the pistol grip) opening at an angle of 75° into a tube of larger volume (corresponding to the pistol barrel). The neck between the bulb and cylinder was made small, and so graduated that each bulb held exactly the same volume of liquid (33.2 cc ± .05 cc). The glycerine was run directly from a gravity bottle into the bulb by means of a suitable delivery tube, thus facilitating the accurate delivery of a given volume of glycerine. An *equal volume* of the acetone solution of known titer was then added, the cylinder mouth stoppered and shaken for a few moments to secure an initial mixing. The mixture was then brought to the desired temperature and shaken for two or three minutes. (It is

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[1] Eastman Research Lab. Practical.

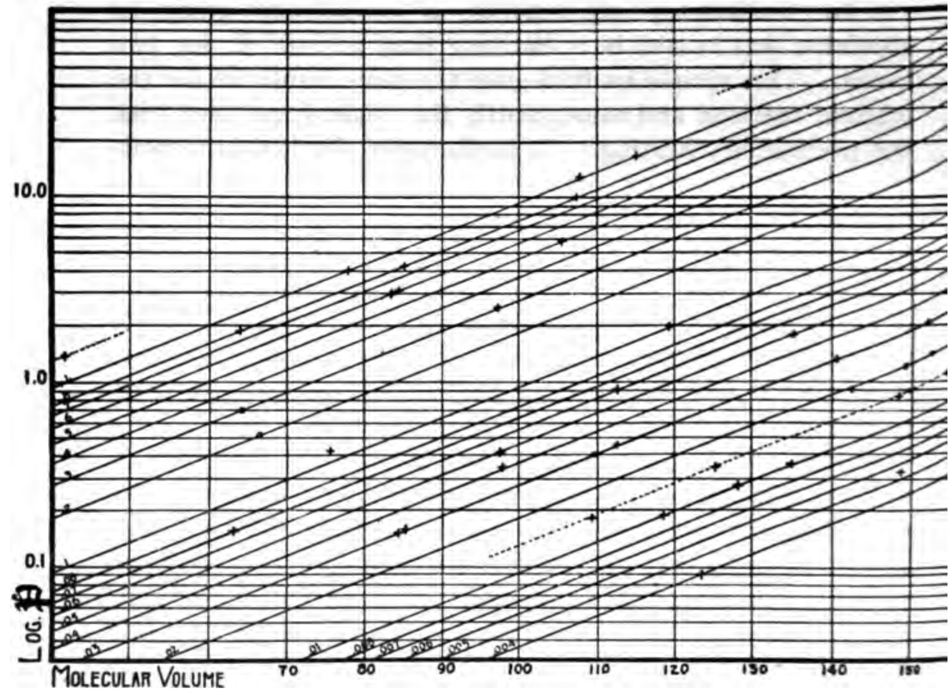

Fig. 1
The Partition Coefficients ($C_2 = 1$ m/L) in the System Acetone: Glycerine

probable that a half-minute's shaking brings the mixture to equilibrium.) After shaking, the acetone, being the liquid of lesser density, can be drawn directly into a pipette inserted through the cylinder mouth. All solutions were made in acetone and titrated with standard aqueous acid or alkali. The concentration in the glycerine was determined by difference.

§ 2. General Considerations

The general picture is the same as it was in the previous systems. It is therefore not necessary to describe it in detail. This system puts the method of correction to a much more severe test, however, because after shaking equal volumes of acetone and glycerine together, the acetone layer loses about 17% in volume, the glycerine increasing 15%, and the total volume decreasing 2%. Thus we are not determining the partition coefficient of the solute between pure acetone and pure glycerine, but between two mixtures of acetone and glycerine. Since the concentration in the glycerine is determined by difference, and on a volume basis, it might be expected that the change in volume which occurs when the two liquids are mixed would introduce a serious complication. The decrease in volume of the acetone layer would necessitate an increase in concentration of the solute, were the solute unable to pass into the glycerine layer. We will see that this actually does take place in some instances. But, where partition does take place, if we deal with concentrations per unit volume of solvent, the facts show that the relative results are not modified. It is probable, however, that the absolute value of the series constants are modified, but this point will not be treated in detail until we consider the relations between the series constants in various systems.

One of the most important points brought out by the data in Table I is the fact that the *absolute values* of the *series constants for the amines and the acids are identical in the system glycerine : acetone*. Consequently the amine and the acids have been considered together in the following tables.

TABLE I

Partition Coefficients of the Amines and Acids in the System Glycerine : Acetone

Series number	a	V_m	C_2	$\mathfrak{p}$ obs.	$\mathfrak{p}$ calc.
Benzilic acid Benzoic acid α-β-Dinitropropionic acid 2.4-Dinitrobenzoic acid Salicylic acid 2.4.6-Trinitrobenzoic acid *n*-Valeric acid		These compounds pass completely into the acetone layer.			
1.414	+1.45154				
Formic acid		41.5	1	1.36	1.39
α-Bromopropionic acid		105.8	10	16.2	16.4
iso-Valeric acid		129.5	1	40.0	40.7
1.0	+1.30103				
Chloroacetic acid		78.0	1	3.90	3.99
n-Butyric acid		107.8	1	12.70	12.52
.8	+1.20412				
Acetic acid		64.0	1	1.85	1.86
Thiacetic acid		73.6	10	2.65	2.69
Propionic acid		85.3	1	4.20	4.22
Dichloroacetic acid		97.2	10	7.0	6.67
Sec-Butyric acid		107.3	1	9.68	9.83
.7	+1.14613				
Propionic acid		85.3	10	3.55	3.69
.6	+1.07918				
Bromoacetic acid		84.5	1	3.13	3.58
.5	+1.00000				
Ethyl amine		64.6	10	1.20	1.20
α-Bromopropionic acid		105.8	1	5.80	5.80
.4	+0.90309				
Thiacetic acid		73.6	1	1.45	1.35
.3	+0.77815				
Ethyl amine		64.6	1	0.71	0.716
Dimethyl amine		66.3	10	0.67?	0.764
Dichloroacetic acid		97.2	1	2.50	2.50
.2	+0.60206				
Dimethyl amine		66.3	1	0.50	0.502
.1414	+0.45154				
Trichloroacetic acid		119.5	10	2.65	2.77
.1	+0.30103				
Trichloroacetic acid		119.5	1	1.95	1.96
.08	+0.20412				

Series number	a	V_m	C_2	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
Anthranilic acid		135.0	10	2.92	2.84
.07	+0.14613				
Fumaric acid		112.6	10	1.02	1.05
Triethyl amine		152.6	10	4.9	4.89
.06	+0.07918				
Diethyl amine		109.3	10	0.81	0.795
Fumaric acid		112.6	1	0.89	0.888
Dipropyl amine		151.5	10	3.75?	4.02
.05	±0.00000				
Malonic acid		97.8	1	0.443	0.427
Anthranilic acid		135.0	1	1.80	1.78
.04	—0.09691				
.03	—0.22185				
Diethyl amine		109.3	1	0.42	0.398
Maleic acid		112.6	1	0.457	0.452
Succinic acid		120.0	1	0.603	0.600
n-Methylpiperidine		133.8	10	0.99	1.02
Bromosuccinic acid		140.5	1	1.25	1.38
Mandelic acid		152.2	1	2.05	2.06
.02	—0.39794				
Piperidine		108.8	1	0.275	2.60
Dipropyl amine		151.5	1	1.20	1.34
Triethyl amine		152.6	1	1.40	1.40
.01414	—0.54846				
o-Phthalic acid		155.6	1	1.09	1.11
.01	—0.69897				
Malic acid		118.8	1	0.192	0.191
Gallic acid		135.0	1	0.352	0.356
.008	—0.79588				
.007	—0.85387				
.006	—0.92082				
Hippuric acid		178.7	1	1.16	1.14
.005	—1.00000				
.004	—1.09691				
Tartaric acid		123.6	1	0.090	0.0918
.0001414	—2.54846				
Dimethyl amine-acid-phthalate		220.1	1	0.128	0.132
.00007	—3.85387				
Trimethyl amine-acid-phthalate		243.6	1	0.157	0.161
.00003	—3.22185				
Piperidine-acid-phthalate		264.4	1	0.155	0.153
Diethyl amine-acid-phthalate		264.9	1	0.158	0.156
.00001414	—3.54846				
n-Methylpiperidine-acid-phthalate		289.4	1	0.180	0.188
Dipropyl amine-acid-phthalate		305.5	1	0.335	0.349
.00001	—3.69897				

TABLE I (*Continued*)

Series number	a	V_m	C_2	𝔓 obs.	𝔓 calc.
Trimethyl amine-acid-phthalate		308.2	1	0.270	0.274
.000005	—4.00000				
Benzylethyl amine-acid-phthalate		328.6	1	0.313	0.299
Di-*iso*butyl amine-acid-phthalate		346.7	1	0.60	0.600
.000003	—4.22185				
Tripropyl amine-acid-phthalate		378.1	1	1.18	1.21
.000001414	—4.54846				
Di-*iso*amyl amine-acid-phthalate		388.1	1	0.816	0.831

	V_m	C_2	𝔓 obs.	a calc.	Corresponding series
Chloroacetic acid	78.0	10	4.45	+1.34836	1.115
Piperidine	108.8	10	0.45	—0.16012	.03458
n-Methylpiperidine	133.8	1	0.57	—0.47413	.01678
Glutaric acid	142.2	1	0.88	—0.42750	.01877
o-Phthalic acid	155.6	10	1.28	—0.48612	.01633
Gallic acid	135.0	1	0.352	—0.74757	.008942

§ 3. The Series Behavior

It was shown in the previous communication that a series must be recognized between Series 1 and Series 2; a value of 1.414 was arbitrarily assigned to this series, and it has been included in the above table. Compounds not falling in the recognized series are given at the end of the table. Note that *n*-methylpiperidine, glutaric acid and phthalic acid give an average value of .01729 which approximates a series corresponding to the √3, or 1.732.

Reference to the following experimental section will show that in many cases the ratio C_A/C_G is constant. For the rest, this ratio increases in about as many cases as it decreases. It is not known whether association in one solvent or dissociation in the other is the predominant factor; but whatever the cause of the inconstant ratio, the interpolation method of obtaining the partition coefficient yields results which are in excellent agreement with those compounds which show a constant distribution ratio, so far as the series behavior is concerned.

The number of compounds occurring in the various series is as follows:

Series	$C_2 = 1\ m/L$	$C_2 = 10\ m/L$
1.414, .1414, etc.	6	2
1.0 .1 etc.	6	0
.8 .08 etc.	3	3
.7 .07 etc.	1	2
.6 .06 etc.	3	2
.5 .05 etc.	6	1
.4 .04 etc.	2	0
.3 .03 etc.	10	2
.2 .02 etc.	4	0

Another important point is that some compounds pass completely into the acetone layer. On the supposition that the fundamental forces involved in this behavior are discontinuous in nature, both as regards the solute and the solvents, it is not surprising that limiting conditions could exist whereby a solute would pass completely in just this way, into one or the other solvent. That is, in a mechanism working by abrupt changes, there must both be a maximum and a minimum activity, and in any system in which one of these limits was reached, the all (unit) or none principal would necessitate complete concentration of the solute in one solvent or the other. Evidence in this direction was seen in the system water: xylene, where it was found that xylene did not extract perceptible amounts of the dibasic acids and other compounds It was thought at that time that this was largely a matter of experimental error, for the predicted partition coefficients were so low that they were difficultly determined. But when the reverse situation is found in this system, i. e., when the solute passes entirely into the organic solvent, it seemed probable that this failure to get a perceptible distribution is a real and not an apparent failure of the distribution law. In general we know that with increasing length in the carbon chain, there comes a time when the solubility in water falls off abruptly; this fact is rendered more intelligible by the recognition of the existence of *abrupt, limiting conditions* in intermolecular relations.

As an example consider the simple aliphatic acids; acetic, propionic, and *sec*-butyric occurring in Series 8, *n*-butyric in Series 1.0 and *iso*-valeric in Series 1.414. One is led to look for *n*-valeric in Series 2, where it would have a partition coefficient of about 59.0, a determinable value. Contrarily, in this and other similar instances the concentration of the solute in the acetone layer is increased (per unit volume) by the diminution in volume of this layer. This increased concentration makes the failure of the distribution law even more apparent.

§ 4. The Acid-Phthalate Salts

The original intention in including some salts of this nature in this study was to determine whether the series constants for the salts would be in agreement with the acids or the amines, or different from both of these. It has been found, however, that the acids and the amines have identical series constants so that this question remains unsettled. It cannot be satisfactorily settled by studying any of the systems previously used containing water, because the partition coefficients in these systems are too small to be determinable with any accuracy. Though little significance can be attached to the fact that the salts do have the same series constants as the amines and acids, it is a very satisfactory way to have the thing work out.

Summary

1. The partition coefficients of fifty acids, amines, and acid-phthalate salts have been determined in the system glycerine : acetone. The results yielded by these data are in excellent agreement with the results obtained in the systems previously studied.[1]

2. A fact not brought out by previous studies is that under certain conditions concerning the nature of the solvents and solute, a solute is not distributed, but is concentrated entirely in one or the other of the solvents.

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[1] Jour. Phys. Chem., 25, 160, 204, 605 (1921).

3. It is a noteworthy point that the series constants for the acids and the amines (and the amine salts of phthalic acid) have the same absolute value in this system, as contrasted with the different values found in systems containing water as one solvent.

Experimental. Section IV

The Partition Coefficients of the Amines, Acids and Acid-Phthalate Salts between Glycerine and Acetone, 25°

The following compounds pass completely into the acetone layer:

- Benzilic acid
- Benzoic acid
- α-β-Dibromopropionic acid
- 2.4-Dinitrobenzoic acid
- Di-*iso*butyl amine
- Salicylic acid
- 2.4.6-Trinitrobenzoic acid
- *n*-Valeric acid

	C_G	C_A	C_A/C_G	*p*
Acetic acid	17.30	29.30	1.69	
	10.00	17.60	1.76	
	4.85	8.60	1.77	
	4.60	8.20	1.78	
	3.875	7.00	1.81	
	2.775	4.775	1.72	
	2.77	4.70	1.70	
	1.6	3.025	1.80	
	1.00	1.825	1.82	*I*
	0.9425	1.725	1.83	1.85
Anthranilic acid	4.60	14.70	3.20	*IO*
	3.20	8.95	2.80	2.92
	1.55	3.80	2.45	*I*
	1.05	2.20	2.09	1.80
Benzylethyl amine-acid-phthalate	10.35	3.65	0.302	
	7.80	2.55	0.327	
	5.45	1.80	0.330	

TABLE (*Continued*)

	C_G	C_A	C_A/C_G	$\mathfrak{p}$
Formic acid	16.0	20.8	1.30	
	5.65	7.8	1.38	
	3.70	5.65	1.39	
	2.0625	2.8875	1.40	Mean
	1.525	2.075	1.36	1.36
Fumaric acid	5.275	5.175	0.981	*IO*
	2.225	2.125	0.954	1.02
	1.3375	1.1875	0.888	*I*
	0.730	0.6350	0.870	0.89
Galic acid	29.85	9.55	0.320	*IO*
	12.85	4.45	0.346	0.318
	6.625	2.325	0.351	*I*
	2.45	0.8625	0.352	0.352
Glutaric acid	9.65	8.15	0.845	
	3.025	2.60	0.860	
	2.950	2.575	0.874	
	1.55	1.350	0.870	*I*
	0.875	0.775	0.885	0.880
Hippuric acid	19.85	22.80	1.15	
	8.20	9.70	1.18	
	4.10	4.80	1.17	Mean
	12.40	2.75	1.15	1.16
Maleic acid	3.05	6.85	0.525	
	7.40	3.50	0.473	
	32.85	1.75	0.454	
	1.50	0.95	0.380	Mean
	1.925	0.4375	0.454	0.457
Malic acid	11.0625	2.2875	0.207	
	5.50	1.10	0.200	
	3.75	0.675	0.180	*I*
	2.60	0.475	0.182	0.192
Malonic acid	14.0	6.05	0.433	
	6.05	2.80	0.462	
	3.775	1.8875	0.500	
	2.4875	1.05	0.423	Mean
	1.20	0.475	0.396	0.443
Mandelic acid	11.6	23.0	1.98	
	8.25	16.45	1.99	
	4.25	8.65	2.04	*I*
	2.075	4.225	2.04	2.05
n-Methylpiperidine	20.8	22.2	1.068	*IO*
	11.05	11.15	1.010	0.99

	C_G	C_A	C_A/C_G	[illegible]
	6.40	6.10	0.954	
	3.50	3.10	0.886	
	2.68	2.06	0.767	
	2.00	1.375	0.687	
	1.73	0.95	0.550	
	1.30	0.65	0.500	*1*
	1.03	0.29	0.282	0.57
n-Methylpiperidine-acid-phthalate	12.75	1.76	0.138	
	4.55	0.90	0.198	
	2.460	0.680	0.276	*1*
	1.280	0.480	0.375	0.180
o-Phthalic acid	6.375	8.025	1.26	*10*
	3.200	3.800	1.19	1.28
	1.5875	1.775	1.12	*1*
	0.8750	0.9375	1.07	1.09
Piperidine	33.3	16.0	0.48	*10*
	23.4	10.6	0.453	0.45
	17.35	7.8	0.45	
	12.00	4.925	0.41	
	9.95	4.0	0.402	
	6.775	2.375	0.350	
	5.45	1.875	0.344	
	3.25	0.85	0.262	
	2.85	0.725	0.254	*1*
	1.3125	0.225	0.144	0.275
Piperidine-acid-phthalate	10.31	1.475	0.143	
	3.86	0.700	0.181	
	1.85	0.482	0.261	
	0.79	0.369	0.467	*1*
	0.356	0.250	0.702	0.155
Propionic acid	9.60	31.00	3.23	*10*
	5.80	19.40	3.34	3.55
	3.50	10.85	3.10	
	2.15	7.45	3.46	
	1.48	5.76	3.89	
	0.78	3.06	3.93	*1*
	0.43	1.74	4.05	4.20
Succinic acid	5.70	3.275	0.575	
	4.725	2.925	0.619	
	2.675	1.550	0.580	
	1.9625	1.325	0.675	Mean
	1.35	0.7625	0.565	0.603?
Tartaric acid	16.25	1.30	0.080	
	16.25	1.25	0.077	

Table *(Continued)*

	C_G	C_A	C_A/C_G	[illegible]
Formic acid	16.0	20.8	1.30	
	5.65	7.8	1.38	
	3.70	5.65	1.39	
	2.0625	2.8875	1.40	Mean
	1.525	2.075	1.36	1.36
Fumaric acid	5.275	5.175	0.981	*IO*
	2.225	2.125	0.954	1.02
	1.3375	1.1875	0.888	*I*
	0.730	0.6350	0.870	0.89
Galic acid	29.85	9.55	0.320	*IO*
	12.85	4.45	0.346	0.318
	6.625	2.325	0.351	*I*
	2.45	0.8625	0.352	0.352
Glutaric acid	9.65	8.15	0.845	
	3.025	2.60	0.860	
	2.950	2.575	0.874	
	1.55	1.350	0.870	*I*
	0.875	0.775	0.885	0.880
Hippuric acid	19.85	22.80	1.15	
	8.20	9.70	1.18	
	4.10	4.80	1.17	Mean
	12.40	2.75	1.15	1.16
Maleic acid	3.05	6.85	0.525	
	7.40	3.50	0.473	
	32.85	1.75	0.454	
	1.50	0.95	0.380	Mean
	1.925	0.4375	0.454	0.457
Malic acid	11.0625	2.2875	0.207	
	5.50	1.10	0.200	
	3.75	0.675	0.180	*I*
	2.60	0.475	0.182	0.192
Malonic acid	14.0	6.05	0.433	
	6.05	2.80	0.462	
	3.775	1.8875	0.500	
	2.4875	1.05	0.423	Mean
	1.20	0.475	0.396	0.443
Mandelic acid	11.6	23.0	1.98	
	8.25	16.45	1.99	
	4.25	8.65	2.04	*I*
	2.075	4.225	2.04	2.05
n-Methylpiperidine	20.8	22.2	1.068	*IO*
	11.05	11.15	1.010	0.99

	C_G	C_A	C_A/C_G	[illegible]
Diethyl amine-acid-phthalate	12.80	1.65	0.129	
	9.975	1.325	0.133	
	6.65	1.05	0.158	
	4.15	0.775	0.187	*1*
	2.0375	0.450	0.221	0.158
Di-*iso*-amyl amine-acid-phthalate	7.85	6.15	0.783	
	6.30	4.80	0.762	
	4.875	3.625	0.744	
	3.075	2.725	0.886	Mean
	1.9375	1.750	0.905	0.816
Di-*iso*-butyl amine-acid-phthalate	9.65	4.45	0.462	
	7.60	3.80	0.500	
	5.85	3.10	0.530	
	3.95	2.10	0.530	*1*
	1.90	1.10	0.580	0.60
Dimethyl amine (anhydrous)	27.8	20.1	0.723	*10*
	15.8	10.6	0.672	0.67
	7.525	4.775	0.635	
	4.325	2.425	0.561	*1*
	2.25	1.125	0.510	0.50
Dimethyl amine-acid-phthalate	14.75	1.70	0.115	
	10.875	1.30	0.119	
	7.80	1.00	0.128	
	5.4875	0.8125	0.148	*1*
	3.725	0.6250	0.168	0.128
Dipropyl amine	2.75	10.1	3.67	*10*
	2.275	8.775	3.64	3.75
	2.025	5.425	2.69	
	1.60	3.85	2.40	
	1.50	2.90	1.93	
	1.325	1.925	1.45	*1*
	0.925	1.30	1.40	1.2
Dipropyl amine-acid-phthalate	10.90	2.45	0.224	
	7.625	1.825	0.239	
	4.525	1.35	0.298	
	2.50	0.8875	0.335	*1*
	1.45	0.600	0.413	0.335
Ethyl amine	8.20	9.76	1.19	*10*
	4.72	4.76	1.01	1.20
	4.55	4.65	1.02	
	2.70	2.25	0.835	
	1.76	1.26	0.715	
	1.625	1.25	0.77	*1*
	1.20	0.67	0.558	0.71

Table (*Continued*)

	C_G	C_A	C_A/C_G	[illegible]
	6.00	0.60	0.100	
	5.77	0.625	0.105	
	3.075	0.425	0.136	
	2.75	0.4125	0.150	*I*
Thiacetic acid	9.40	30.10	3.20	0.090
	4.60	14.30	3.10	*IO*
	3.60	9.40	2.62	2.65
	2.36	5.36	2.27	
	1.56	3.04	1.95	*I*
Trichloroacetic acid	1.70	20.90	12.30	1.45
	1.25	7.70	6.17	*IO*
	1.075	5.40	5.02	7.55
	0.7625	2.8375	3.72	
	0.600	1.50	2.50	*I*
Triethyl amine	3.20	25.80	8.06	1.95
	2.25	13.20	5.87	*IO*
	1.65	6.35	3.85	4.9
	1.175	2.875	2.44	
	0.865	1.470	1.70	*I*
Triethyl amine-acid-phthalate	12.775	2.70	0.2115	1.40
	9.35	2.00	0.214	
	5.60	1.45	0.259	
	4.075	1.10	0.270	*I*
	2.35	0.70	0.398	0.270
Trimethyl amine-acid-phthalate	13.55	1.95	0.144	
	10.35	1.50	0.145	
	5.65	0.90	0.159	*I*
	4.20	0.70	0.167	0.157
Tripropyl amine-acid-phthalate	6.80	4.25	0.625	
	4.45	3.125	0.702	
	2.55	2.125	0.843	*I*
	1.375	1.400	1.020	1.18
iso-Valeric acid	0.500	23.00	46.0	
	0.300	10.30	34.4	
	0.1375	5.15	37.4	Mean
	0.1125	4.75	42.3	40.0

School of Hygiene and Public Health
Dept. of Physiology, Baltimore, Md.

THE NATURE OF SECONDARY VALENCE

BY HOMER W. SMITH

PART V. PARTITION COEFFICIENTS IN SYSTEMS CONTAINING WATER AS ONE COMPONENT WITH SPECIAL REFERENCE TO THE ABSOLUTE VALUES OF THE SERIES CONSTANTS

1. Introduction

We have thus far considered the behavior of various solutes in the systems:

Water : xylene
Water : chloroform
Water : ether
Glycerine : acetone

By applying the equation:

Log 100[illegible] $= V_m/60.00 \pm a$

we have derived values for *a* which we have arbitrarily called series constants. The antilogs of these series constants for various solutes in each system have been shown to be related to each other by simple proportion. The series constants of the various systems containing water also appear to be similarly related. That is, if we denote the series constant for any given series in xylene by a_x, ether a_e, chloroform a_c, then:

Acids	Amines
$a_e = a_x + \log\ 15.00$	$a_x + \log\ 1.50 + 0.58840$
$a_c = a_x + \log\ 5.00$	$a_x + \log\ 5.0 + 0.58840$

In view of the simple proportion existing between these three systems, it seemed important to extend the investigation to a larger number of solvents, to see if this rule of simple proportionality is universally true.

It has been said that the behavior of a given solute in two phase systems such as these is determined not only by the nature of the solute, but by the nature of the solvents, as well. Nearly all the acids, and a few of the amines, have been observed to change their relative behavior (or series number) in various systems. This uncertainty about the series number of a given solute in any system makes it essential to determine the partition coefficients for several solutes before the series constants for a given system can be safely evaluated. But when several series are represented in the experimental data, the series constants for any system can be determined with certainty because there are a limited number of series and they can be identified by their numerical inter-relationships.

Consequently the literature was carefully searched for material which would aid in obtaining the series constants for various solvents when used with water, other than the three already discussed (xylene, chloroform and ether). Such data as are available, together with some original data, are given in this paper.

2. The Corrected Series Constants

Before entering into the discussion of these other systems, it is necessary to point out that the series constants for the systems already discussed have been modified, in the light of subsequent information, with a view to increased accuracy.

In correcting them, I have been guided by several more or less obvious, but nevertheless assumptive, relationships. In the first place, the series constants for the amines appear to differ from the series constants of the acids by a constant amount (viz., 0.58840). This fact has not been previously mentioned and will be discussed later on. Secondly, in both xylene and ether, one amine series appears to extrapolate to a unit value at $V_m = 0$ (viz., for Series .6 in xylene and .4 in ether, $a = \pm 0.00000$). Thirdly, the series constants of the acids in the cases of xylene, chloroform and ether, as I have already pointed out, are apparently related to each other by pure integrals (viz., log 1.0 : log 5.0 : log 15.0).

I have assumed that these relationships actually did exist. Thus it has been possible to correlate the values derived in all three systems, and to evaluate the series constants with much greater accuracy. If these assumptions are correct, and if the simple numerical relationships exist between the various series in any system as we suppose, it is evident that the series constants for the integral series, at least, can be directly evaluated with ultimate accuracy to any degree.

The series constants in the system water : xylene, as originally given, were derived from the experimental data by a cut-and-try process, since here there is no series which on extrapolation approximates any unit value at $V_w = 0$; the constant difference law between the acids and amines was not at that time recognized. Consequently it occasioned some surprise when, on reworking the data, I found that these values were fairly accurate to the fourth decimal place.

That the foregoing assumptions, by which I have been guided in revising the series constants, are correct is borne out by the study of other solvents presented in this paper.

In theory, since all series in any system, and since all systems as well, are related to each other by simple proportion it is only necessary to know one series constant to obtain the series constant for any series in any system. But for the sake of clarity, I have chosen the series constants for the system water : xylene for presentation. One might suppose that an infinite number of series could exist; but the evidence indicates that the number of series in each system is limited. It is impossible to be sure about this at the present time, but since some method of numbering the various series is necessary, a purely arbitrary designation has been adopted. They have already been numbered by integers from 1 to 10; that series having the highest value of *a* in any system has now been designated with an integer, and the succeeding series by decimation. This aids in their indentification since they repeat themselves after every decrease of ten times in the partition coefficient. I have chosen Series 4 as a beginning in the following table because it is the highest one so far observed among the acids or amines.[1]

In résumé, it may be said that these series comprise the recognized possible values of $\mathfrak{P}$; the partition coefficients in several series are in the same proportion to each other as either the respective series numbers, or the antilogs of the respective series constants, a.

TABLE I

The Corrected Series Constants for the Acids in the System Water : Xylene

Series number	a	Series number	a
4.	+0.23551	.4	—0.76449
3.	+0.11057	.3	—0.88943
2.	—0.06552	.2	—1.06552
1.414	—0.21610	.1414	—1.21610
1.	—0.36655	.1	—1.36655
.8	—0.46346	.08	—1.46346
.7	—0.52145	.07	—1.52145
.6	—0.58840	.006	—2.58840
.5	—0.66758	.005	—2.66758

etc.

3. The Series Constants of Various Systems in which Water is One Component

Instead of tabulating the data as actually found, I have presented the "pure" or theoretical values indicated, and then calculated the values of $\mathfrak{P}$ on the basis of these "pure" values. Thus I have ascertained that the series constant for toluene is a_x + log 0.0000 (still an assumption, of course) and used this "pure" relationship in calculating the desired values of $\mathfrak{P}$. This enables us to avoid in discussion the errors arising in the experimental work, and presents the data in a final form. The degree of agreement between $\mathfrak{P}$ obs. and $\mathfrak{P}$ calc. indicates the degree to which the assumption of simple proportionality is borne out. The figures given under the caption C_2 show the concentration in the second solvent to which interpolation is made to obtain $\mathfrak{P}$.

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[1] In comparing two systems in this manner we have each series characterized; there will be a Series 1.0 in each system, a Series .1, etc. That these series are chemically identical, or rather chemically related, is evident from the facts that one compound (such as diethylamine) will appear in the same series in all systems, and that the various systems are related, through any one series, by simple proportion.

TABLE II
Water : Toluene
Acids. $a_t = a_x \pm 0.00000$

Series number	a	V_m	C_2	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
14.14	+0.78390				
	Phenol	101.9	1000.0	2.75[1]	3.03
.8	—0.46346				
	Chloroacetic acid	78.0	1000.0	0.07[1]	0.069
.6	—0.58840				
	sec-Butyric acid	107.3	1.0	0.16	0.1585
.3	—0.88943				
	Chloroacetic acid	78.0	100.0	0.028[1]	0.0257
.2	—1.06552				
	Acetic acid	64.0	1.0	0.0101[1]	0.010
	Amines. $a_t = a_x + 0.58840$				
4.0	+0.82391				
	Pyridine	89.5	100.0	2.06[1]	2.07
1.0	+0.22185				
	Trimethylamine	88.0	100.0	0.43[1]	0.488

TABLE III
Water : Benzene
Acids. $a_b = a_x + \log 1.25$

Series number	a	V_m	C_2	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
6.0	+0.50851				
	Benzoic acid	126.9	10.0	4.20	4.23
2.0	+0.03139				
	Benzoic acid		1.0	1.45	1.41
1.0	—0.26964				
	Acetone	76.6	1000.0	0.98	1.00
.8	—0.36655				
	Acetic acid	64.0	100.0	0.054[1]	0.050
.6	—0.49149				
	sec-Butyric acid	107.3	1.0	0.202	0.198
.1	—1.26964				
	Picric acid	172.0	100.0	4.0	3.95
.04	—1.66758				
	Picric acid		10.0	1.55	1.582

TABLE III (*Continued*)

Amines. $a_b = a_x + \log 1.25 + 0.58840$

4.0	+0.92082 Pyridine	89.5	100.0	2.6[1]	2.565
.8	+0.22185 Trimethylamine	88.0	100.0	0.50[1]	0.488
.4	—0.07918 Diethylamine	109.3	1.0	0.560	0.553

TABLE IV

Water : Carbon Tetrachloride

Acids. $a_a = a_x + \log 1.414$

Series number	*a*	V_m	C_2	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
8.	+0.68705 Phenol	101.9	1000.0	2.45[1]	2.429
5.	+0.48293 Acetone	76.6	1000.0	0.61	0.575
4.0	+0.38602 Acetone	76.6	100.0	0.450[1]	0.460
1.414	—0.06559 Acetic acid	64.0	1000.0	0.10[1]	0.100
.5	—0.51707 Acetic acid	64.0	100.0	0.0365[1]	0.0354
	Chloroacetic acid	78.0	1000.0	0.06[1]	0.0606
.3	—0.73892 *sec*-Butyric acid	107.3	1.0	0.104	0.112
.1	—1.21604 Chloroacetic acid	78.0	100.0	0.0115[1]	0.0121

Amines. $a_a = a_x + \log 1.414 + 0.58840$

.4	—0.02558 Diethylamine	109.3	1.0	0.615	0.625
.007	—1.78254 Ammonia	26.9	10.0	0.0047	0.0046

Nearly nine-tenths of the data incorporated in the foregoing tables have been obtained from the literature. Moreover everything available that could possibly be utilized has been used. It is indeed remarkable that such satisfactory agreement should be secured from the experimental work of so many investigators.

TABLE V

Water : Amyl Alcohol

Acids. $a_a = a_x + \log 5.00$

Series number	a	V_m	C_2	p obs.	p calc.
14.14	+1.48287				
	Phenol	101.9	100.0	15.7	15.18
4.0	+1.93448				
	Acetic acid	64.0	1000.0	0.921	1.00
.5	+1.03149				
	Picric acid	172.0	10.0	7.4	7.91
.4	+0.93448				
	Picric acid		100.0	6.7	6.325
.1	+0.33242				
	Oxalic acid	75.6	100.0	0.40	0.422
.08	+0.23551				
	Oxalic acid		10.0	0.295	0.313
.03	−0.19046				
	Succinic acid	120.0	100.0	0.675	0.6324
Amines. $a_a = a_x + \log 5.00 + 0.58840$					
1.0	+0.92082				
	Triethylamine	152.6	100.0	26.0	29.1
.8	+0.82391				
	Methylamine	43.0	100.0	0.335	0.347
.6	+0.69897				
	Ammonia	26.9	100.0	0.146	0.140
.4	+0.52288				
	Diethylamine	109.3	1.0	2.22	2.21

The primary motive in presenting these data is to show that a simple proportion exists between solvents, as well as between solutes. That all the proportions are not simple, or integral, is not a matter to concern us greatly at the present time. It may be remarked that they have been determined with sufficient accuracy to warrant careful consideration. Consider, for example, carbon tetrachloride; here,

$$a_d = a_x + \log\ 1.414$$

With the exception of two instances, the data given for this system were determined by Herz and Lewy. The mean algebraic variation between the observed and calculated values is +0.013. The maximum variation is +0.075

in the case of diethylamine, one of my own determinations. The existence of series corresponding to 1.414 has already been discussed and it was pointed out that this value was chosen because it is the square-root of two.[1]

TABLE VI

Water : Bromoform

Acids. $a_{br} = a_x + \log 5.0$

Series number	a	V_m	C_2	$\mathfrak{P}$ obs.	$\mathfrak{P}$ calc.
4.0	+0.93448				
	Phenol	101.9	1000.0	4.3[2]	4.294
.6	+0.11057				
	Acetic acid	64.0	1000.0	0.148[2]	0.1502
.2	—0.36655				
	Chloroacetic acid	78.0	1000.0	0.094[2]	0.108
.1	—0.66758				
	Picric acid	172.0	100.0	1.6[2]	1.582
	Amines. $a_{br} = a_x + \log 5.0 + 0.58840$				
.01414	—0.92873				
	Ammonia	26.9	100.0	0.0292	0.033

4. The Use of Diethylamine in Determining the Series Constants in Various Systems

If we could be sure that a given compound would never shift from one series to another, it would only be necessary to study that one compound in order to obtain the series constants for any system.

It was noticed, however, in the first three water-containing systems studied that the acids were likely to occur in different series in each system. The amines nearly always appeared in the same series in every system. This regular behavior of the amines persists in the systems studied above, though there are exceptions.

Diethylamine is one compound which has always appeared in the same series (i. e., Series .4) and for this reason it has been studied in a number of systems where only a small quantity of the organic solvent was available or where time prohibited extensive work. The results are listed below. It cannot be said definitely that diethylamine has not changed its series number in any of these systems, but from its previous behavior I believe this to be unlikely. On this assumption we then have this simple relationship between xylene and the unevaluated solvent, *r*:

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[1] Jour. Phys. Chem., **25**, 611 (1921).

[2] For the derivation of these values see Section I of Part II of this series. Jour. Phys. Chem., **25**, 204 (1921). For all other values see the following experimental section.

$$\frac{\text{Antilog } a_r}{\text{Antilog } a_x} = \frac{\mathfrak{P}_r}{\mathfrak{P}_x}$$

That is, the series constants of the two systems bear the same proportion as do the partition coefficients of diethylamine. Of course $\mathfrak{P}$ must be obtained from equal values of C_2. These data are all based upon determinations of $\mathfrak{P}$ when C_2 is 1 *m*/L.

TABLE VII

The Behavior of Diethylamine in Various Systems

	$P_r [C_2 = 1\ m/L.]$	Observed ratio[1] P_r/P_x	Pure ratio P_r/P_x
Paraffin Oil	0.185	0.419	.4?
Petroleum (C_9–)	0.277	0.627	.625?
Trichlorobenzene	0.333	0.754	.75
iso-Amylphenyl ether	0.435	0.985	1.00
Bromobenzene	0.556	1.26	1.25
o-Dichlorobenzene	0.650	1.47	1.50
n-Butylbromide	0.65	1.47	1.50
n-Butyl ether	0.65	1.47	1.50
Ethylene chloride	0.884	2.00	2.00
sec-Octyl alcohol	1.11	2.52	2.50
n-Butyl alcohol	2.20	4.98	5.00
iso-Butyl alcohol	2.20	4.98	5.00

We now have the series constants for twenty systems containing water. Eight of these have been determined from data based on several solutes (these are marked with an asterisk in the following table). The remaining twelve are based on diethylamine alone.

---

[1] $P_x = 0.442$

TABLE VIII
Summary of Series Constants

Water and	a (Acids)	a (Amines)
Paraffin Oil	$a_x+\log 0.40$	$+0.58840$
Petroleum	$a_x+\log 0.625$	$+0.58840$
Trichlorobenzene	$a_x+\log 0.75$	$+0.58840$
Xylene*	given as a_x	$+0.58840$
iso-Amylphenyl ether	$a_x+\log 0.00$	$+0.58840$
Toluene*	$a_x+\log 0.00$	$+0.58840$
Benzene*	$a_x+\log 1.25$	$+0.58840$
Bromobenzene*	$a_x+\log 1.25$	$+0.58840$
Carbon tetrachloride*	$a_x+\log 1.414$	$+0.58840$
Dichlorobenzene	$a_x+\log 1.50$	$+0.58840$
n-Butylbromide	$a_x+\log 1.50$	$+0.58840$
n-Butyl ether	$a_x+\log 1.50$	$+0.58840$
Ether[1]*	$a_x+\log 15.00$	$+\bar{1}.58840$
Ethylene chloride	$a_x+\log 2.00$	$+0.58840$
sec-Octyl alcohol	$a_x+\log 2.50$	$+0.58840$
n-Butyl alcohol	$a_x+\log 5.00$	$+0.58840$
iso-Butyl alcohol	$a_x+\log 5.00$	$+0.58840$
Amyl alcohol*	$a_x+\log 5.00$	$+0.58840$
Chloroform*	$a_x+\log 5.00$	$+0.58840$
Bromoform*	$a_x+\log 5.00$	$+0.58840$
Glycerine : acetone	$a_x+1.66758$	±0.00000

5. The Behavior of Acids and Amines

A very interesting point brought out in the above table is the fact that the series constants of the amines and the acids differ by a constant amount ($+0.58840$) in those systems which contain water, while there is no difference in the system glycerine: acetone.

I was led to wonder while preparing the data on xylene and chloroform why the amines should behave differently, so far as the absolute values of the series constants are concerned, from the acids; it now appears that this difference is to be attributed to the presence of water in the system, for the difference disappears in a water-free system. No satisfactory explanation of this important relationship has occurred to me as yet, but it may be worth while to make this comment: The value +0.58840 corresponds to an increase in molecular volume of 35.14 units. This is approximately the volume of two molecules of water at 25° (18.03 × 2 = 36.06). The molecular volumes of the amines have been figured on the basis of unhydrated molecules; that is, as RNH_2, etc., and not RNH_3OH. In comparing the amines with the acids there is no doubt but that this chemically combined molecule of water should be included in the calculation of the volume.

---

[1] Note that the anomaly in the system water : ether appears to rest with the acids, the series constants of which are ten times as great as the series constants of the amines. In so far as the amines are concerned, this system ($a_x+\log 1.50$) falls into a position relative to the other systems which is in agreement with its general physical properties. This suggests that other systems (*n*-butyl ether, etc.) might show a similar discrepancy if studied with the acids as well as the amines, but at the time of writing it has been impossible to cover these points.

But why another molecule of water should be involved is not so easily explained, unless we assume that all amines possess in aqueous solution one molecule of water of hydration. Such an assumption is favored by a great number of facts indicating that the amines are hydrated to a much greater extent than the acids or other compounds, but that this water of hydration is constant in amount and equal to one molecule is not so easily believed.

Summary

1. The series constants for eight systems in which water is one component have been derived from data in large part quoted from the literature.
2. In each of these systems, the series constants for the various *solutes* studied are related to each other by simple proportion.
3. The series constants for twelve systems in which water is one component have been tentatively formulated through the study of diethylamine.
4. In all twenty of the systems studied, the series constants for the various *systems* are related to each other by simple proportion.
5. In every system in which water is one component, the amines differ from the acids in the value of their series constants by a constant amount.

6. It is concluded that the intermolecular forces concerned in the solvent powers of various molecular species differ in intensity by discrete and simply related amounts. The bearing of the foregoing data on the structure of the atom and on the quantum theory will be discussed in the next communication.

EXPERIMENTAL

C_1 : Concentration in Water Layer.
C_2 : Concentration in Other Layer.

The figures given in the last column show the concentration of C_2 to which interpolation for $\mathfrak{P}$ is made. All concentrations in millimols per liter.

		C_1	C_2	C_1/C_2	$\mathfrak{P}$
Amyl alcohol					
Acetic acid	20°	1326.0	1208.0	0.911	H. and F.
		1024.5	948.1	0.925	
		794.4	729.6	0.918	
		351.5	330.4	0.941	*1000*
		88.88	80.34	0.909	0.921
Ammonia	20°	1970.0	287.4	0.146	H. and F.
		1016.5	154.9	0.151	
		579.5	84.22	0.145	
		364.06	54.55	0.149	
		240.83	34.61	0.144	*100*
		89.17	12.24	0.137	0.146
Diethylamine	25°	4.95	20.00	4.04	S
		2.875	10.625	3.70	
		1.675	5.450	3.26	
		1.05	2.9625	2.82	*1*
		0.65	0.625	2.52	2.22
Methylamine	25°	1061.3	397.4	0.374	H. and F.
		812.6	298.1	0.367	
		642.9	231.5	0.360	
		505.4	175.9	0.348	
		303.6	107.0	0.352	*100*
		115.5	38.04	0.330	0.335
Oxalic acid	20°	1002.9	430.0	0.47	H. and F.
		827.9	340.0	0.47	
		669.9	255.0	0.43	*100*
		459.9	171.7	0.43	0.40
		236.4	72.33	0.38	
		127.5	32.73	0.34	*10*
		68.06	14.51	0.31	0.295
Phenol	25°	390.0	5630.0	14.4	H. and F.
		383.0	5410.0	14.1	

EXPERIMENTAL (*Continued*)

	C_1	C_2	C_1/C_2	[illegible]
	16.0	260.0	16.2	
	7.0	110.0	15.7	*100*
	4.7	75.0	16.0	15.7
Picric acid 25°	64.23	254.9	6.50	H. and F.
	44.71	163.8	6.65	
	31.61	107.9	6.90	*100*
	18.69	51.82	6.82	6.7
	9.203	18.50	7.10	*10*
	5.531	9.296	10.07	7.4
Succinic acid 20°	1155.5	711.9	0.616	H. and F.
	809.9	519.95	0.642	
	404.95	271.47	0.670	
	103.73	70.77	0.682	*100*
	26.84	18.80	0.703	0.675
Triethylamine 25°	27.74	741.8	26.0	H. and F.
	16.64	440.8	26.5	*100*
	8.75	227.3	26.0	26.0
iso-Amylphenyl ether				
Diethylamine 25°	9.50	3.90	0.411	S.
	5.775	2.55	0.442	
	3.30	1.35	0.410	*1*
	1.85	0.815	0.440	0.435
Benzene				
Acetone 25°	2216.7	2394.7	1.080	H. and F.
	1936.7	2071.7	1.070	
	1208.3	1204.5	0.997	
	918.5	896.7	0.976	
	608.9	575.7	0.945	*1000*
	220.0	206.5	0.938	0.980
Benzoic acid 25°	4.00	19.75	4.93	S.
	2.80	11.90	4.25	
	1.725	5.50	3.19	*10*
	1.300	3.45	2.57	4.20
	0.925	1.95	2.11	*1*
	0.8725	1.525	1.75	1.45
sec-Butyric acid 25°	18.90	5.0	0.264	S.
	9.60	2.20	0.230	
	3.75	0.7125	0.190	*1*
	2.55	0.410	0.170	0.202
Diethylamine 25°	22.30	18.30	0.822	S.
	11.95	9.05	0.756	
	6.575	4.35	0.662	
	3.875	2.475	0.638	*1*
	2.1375	1.2375	0.578	0.560
Picric acid	26.09	94.01	3.60	K.

EXPERIMENTAL (*Continued*)

		20.80	77.90	3.74	*100*
		18.82	61.84	3.28	4.0
		13.20	35.90	2.72	*10*
		9.73	19.77	2.03	1.5
Bromobenzene					
Diethylamine	25°	7.15	4.05	0.567	S.
		3.825	2.075	0.542	
		3.40	1.70	0.50	
		2.1875	1.1375	0.520	
		2.0875	1.225	0.587	
		1.4625	0.8875	0.607	(mean)
		1.100	0.625	0.5680	0.556
Bromoform					
Ammonia	25°	8530.0	417.0	0.0489	H. and L.
		8050.0	362.0	0.0449	
		6300.0	235.0	0.0370	
		4180.0	131.0	0.0132	*100*
		2590.0	70.0	0.0272	0.0292
n-Butyl alcohol					
Diethylamine	25°	2.50	6.8	2.72	S.
		1.35	3.175	2.35	
		0.675	1.540	2.30	*1*
		0.275	0.575	2.09	2.2
iso-Butyl alcohol					
Diethylamine	25°	1.0875	2.6125	2.4	S.
		0.650	1.500	2.3	
		0.550	1.225	2.22	*1*
		0.300	0.625	2.08	2.20
n-Butyl bromide					
Diethylamine	25°	6.60	4.00	0.606	S.
		3.475	2.20	0.633	
		2.10	1.3375	0.637[1]	
		1.10	0.725	0.660	0.65
n-Butyl ether					
Diethylamine	25°	7.75	4.55	0.587	S.
		4.05	2.45	0.605	
		2.1625	1.375	0.632	*1*
		0.925	0.625	0.676	0.65
Carbon tetrachloride					
Acetone	25°	2870.0	2100.0	0.733	H. and L.
		1660.0	997.0	0.601	*1000*
		1010.0	514.0	0.511	0.61
		322.0	146.0	0.454	*100*
		186.0	83.3	0.449	0.45
Ammonia	25°	8590.0	73.5	0.00856	H. and L.
		6860.0	46.4	0.00676	

EXPERIMENTAL (*Continued*)

		C₁	C₂	C₁/C₂	p
		5700.0	35.7	0.00626	
		2350.0	11.8	0.00502	*10*
		1730.0	7.87	0.00455	0.0047
sec-Butyric acid	25°	17.50	2.70	0.154	S.
		13.80	1.725	0.125	
		9.25	1.05	0.1135	
		7.10	0.595	0.0838	*1*
		4.775	0.305	0.0639	0.104
Diethylamine	25°	7.80	7.00	0.897	S.
		5.075	4.00	0.788	
		2.525	1.9375	0.700	*1*
		1.425	0.850	0.597	0.615
o-Dichlorobenzene					
Diethylamine	25°	11.70	7.15	0.61	S.
		5.50	3.30	0.60	
		3.175	2.325	0.732	
		1.55	1.075	0.693	(mean)
		1.475	0.95	0.643	0.650
Ethylene chloride					
Diethylamine	25°	4.475	4.075	0.910	S.
		2.15	1.925	0.886	
		1.1875	1.05	0.886	*1*
		0.95	0.835	0.880	0.884
sec-Octyl alcohol					
Diethylamine	25°	2.10	2.50	1.19	S.
		1.75	2.01	1.16	
		1.05	1.20	1.14	*1*
		0.475	0.50	1.05	1.11
Paraffin oil					
Diethylamine	25°	16.10	3.00	0.186	S.
		8.80	1.50	0.170	
		8.70	1.40	0.161	
		5.25	1.025	0.192	
		3.35	0.650	0.194	
		3.30	0.650	0.1955	
		3.20	0.575	0.180	(mean)
		1.475	0.300	0.204	0.185
Petroleum (B. P. 145° –155° (Nonane))					
Diethylamine	25°	11.35	3.25	0.287	S.
		7.25	2.05	0.283	
		4.825	1.425	0.295	
		4.50	1.30	0.289	
		4.175	1.275	0.305	
		2.7125	0.850	0.313	

EXPERIMENTAL (*Continued*)

	1.9725	0.450	0.228	(mean)
	1.6375	0.3625	0.222	0.277
Toluene				
sec-Butyric acid 25°	15.70	5.50	0.350	S.
	8.90	1.65	0.185	
	3.60	0.50	0.139	*1*
	1.975	0.2025	0.1025	0.160
1,3,4-Trichlorobenzene				
Diethylamine 25°	9.45	4.05	0.43	S.
	4.95	1.925	0.403	
	3.325	1.125	0.346	*1*
	1.4375	0.4125	0.287	0.333

H. and F.—Herz and Fischer: Ber. deutsch. chem. Ges., **37,** 4746 (1909); **38,** 1138 (1905).

H. and L.—Herz and Lewy: Zeit. Elektrochemie, **11,** 818 (1905); Sitzungsber. schles. Ges. vaterländische Kultur. Natur-Wissenschaftl. Sektion, **1906.** Most of these data are also given by Herz; Sammlung Chemischer und chemisch-technischer Vortrage, **15,** 1 (1910–11).

K.—Kuriloff: Zeit. phys. Chem., **25,** 418 (1898).

S.—Original.

Johns Hopkins University
School of Hygiene and Public Health
Department of Physiology
Baltimore
and
Lilly Research Laboratories
Eli Lilly and Company
Indianapolis

THE NATURE OF SECONDARY VALENCE

BY HOMER W. SMITH

Part I	Preliminary Communication,	Jour. Phys. Chem., **25,** 160 (1921).
Part II	Systems water: xylene and water: chloroform,	Ibid., **25,** 204 (1921).
Part III	System water: ether,	Ibid., **25,** 605 (1921).
	Supplementary note on extrapolation method of correction,	Ibid., **25,** 616 (1921).
Part IV	System glycerine: acetone,	Ibid., **25,** 721 (1921).
Part V	Systems containing water and paraffin oil; petroleum; tri-chlorobenzene; *iso*-amyl phenyl ether; toluene; benzene; bromo-benzene; carbon tetrachloride; dichlorobenzene; *n*-butyl bromide; *n*-butyl ether; ethylene chloride; *sec*-octyl alcohol; *n*-butyl alcohol; *iso*-butyl alcohol; amyl alcohol and bromoform,	Ibid., **26,** 256 (1922).

VI. Summary and Discussion

I. Summary

This investigation was prompted by the desire to ascertain something of the nature of the forces involved in liquid solutions. To this end the distribution of various organic solutes between immiscible solvents was chosen as the most promising and direct experimental procedure. In all, about twelve hundred final determinations have been presented, involving twenty-one systems each consisting of two immiscible liquids.

These results as they stand are not comparable with one another because of the complications introduced by dissociation, association and the mutual solubility of solvents. It has been shown, however, that these complications can be eliminated in part by an empirical method of correction, and the partition coefficients so obtained put on a mathematically comparable basis, even though their physical-chemical significance is still obscure. This method of correction consists in determining by interpolation the partition coefficient when the concentration in one solvent has a fixed arbitrary value. The interpretation of the results obtained by means of this method of correction is independent of the arbitrary value chosen for interpolation.

The principal points established by a consideration of the experimental data are as follows:

When the partition coefficients of various substances for any one system are compared on a basis of molecular volume, it is found that they may be covered by the equation

$$\text{Log } 100\ \text{[illegible]} = \frac{V_m}{60.00} \pm a$$

where V_m is the molecular volume at the boiling point.

A limited number of values of *a* are required to cover the behavior of all substances in any one system. (All substances covered by the same value of *a* in any one system have been called a "series," and the corresponding values of *a* the "series constants.")

The various values of *a* (or series constants) in any one system have been found to be related to each other by simple proportion.

It has also been found that the series constants for any one series in twenty systems containing a common solvent (water) are related to each other by simple proportion.

The above facts are interpreted as indicating that solubility is a function of the molecular volume of the solute. It is believed that the stepwise phenomena of series, as shown by the integrally related values of *a* in the above equation, are attributable to discrete or abrupt variations in the intensity of the intermolecular forces associated with various molecular species.

2. Homogeneity and Three-Dimensional Symmetry

Before discussing the fundamental significance of these results, it will be necessary to consider again certain points, already discussed in Part I, disclosed by molecular volume relations. It has been said that the experimental facts relating to the molecular volumes of liquid chemical compounds indicate that every atom contributes a definite volume to the molecular volume. Some atoms vary in their atomic volumes as a result of constitutive influences (that is, interactions attributable to the chemical arrangement of the atoms in the molecule) but these constitutive variations in atomic volume are well defined and are related in no way to what we may conceive as spatial configuration. A consideration of molecular volume relations shows that the configurations suggested in structural formulae indicate only the chemical properties of the constituent atoms in the molecule, and in no way define the actual spatial relationships of the various parts or of the whole. The molecular volume is the simple sum of the volumes of the constituent atoms, and there is no additional space which can be attributed to interatomic or intermolecular crevices resulting from fixed spatial configurations as suggested by these formulae. To repeat the example previously used, there is no hole inside the benzene molecule or cracks or crevices between the atoms, as there would surely be if the molecule consisted of rigid spheres of carbon with smaller rigid spheres of hydrogen tacked on at various places. The principle of the perfect sum implies that the six carbons and their attendant hydrogens make up a domain which has the same shape, in the electro-dynamic sense, as has each of the components, much as though we had put several little spheres together to make one big sphere.

We have seen in the data presented in these papers that molecular volume appears to be one of the factors which determine the behavior of various molecular species in homogeneous liquid systems. This determinative rôle of molecular volume is independent of the chemical nature of the molecular species involved and constitutes new evidence of a most conclusive kind in regard to the shapes of molecules. We may summarize this evidence as follows: substances in the homogeneous liquid phase behave toward their environment as though every molecule homogeneously filled a space which is symmetrical in three dimensions.[1]

An assumption somewhat of this nature has been made from time to time by mathematicians when considering the attraction of one molecule for another, but the chemist, in the absence of direct evidence to the contrary, has persistently clung to notions of chemical configuration and spatial relationships.[2] The mathematician speaks of point-to-point action, and avoids the difficulties arising from chemical considerations by arguing that this alleged point-to-point action is only a statistical result. As to how far this is so, it is hard to say because of course we cannot avoid statistical observation and we do not know the exact extent to which the real molecular domain is increased by the translational motion of heat. But the specific nature of the optical properties, the compressibilities and more especially the radiation of heat from liquids and solids indicate that only a small fraction of the apparent molecular domain is to be attributed to translational motion. It seems instead that when there is an expansion of the molecular domain on the absorption of energy, it is to be attributed to an actual expansion of the size of the molecule.[3]

But even if we agree to subtract a certain amount from the observed molecular volume to allow for space taken up by translational motion, we must still apply the principle of homogeneity and three-dimensional symmetry to the remaining real volume, for otherwise molecular volume could not function as a determinative factor in solubility in the precise manner in which it does. So taking into account the additive nature of molecular volume and the rôle of molecular volume in homogeneous liquid solutions, we must look upon this homogeneity and three-dimensional symmetry of liquid molecular domains as an actual fact and not an apparent one. We must picture the electrons of the atoms and molecules which make up a mass of liquid as filling, by their electrodynamic activity, the entire liquid domain, being at equilibrium at all points, and leaving no crevices or dead-spaces wherein other electrons or atoms or molecules can be inserted without disturbing this equilibrium.

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[1] Attention is called to the phrase "in the homogeneous liquid phase." The work of Langmuir (Jour. Am. Chem. Soc., **39,** 1848 (1917)) and Harkins and his collaborators (Ibid., **39,** 541 (1917)) shows that molecules at the surfaces of liquids are oriented and possess more or less definite shapes, but neither Langmuir's nor Harkins' work applies to molecules situated within a homogeneous liquid where the surrounding field of force is symmetrical.

[2] Among others who have argued from this premise are S. C. Bradford: Phil. Mag., **38,** 696 (1919); J. J. Thomson: **27,** 757 (1914); W. C. McC. Lewis: **28,** 104 (1914); W. D. Harkins and H. H. King: Jour. Am. Chem. Soc, **41,** 970 (1919).

[3] See Gervaise LeBas: "The Molecular Volumes of Liquid Chemical Compounds," p. 254 (1915).

3. The Nature of the Intermolecular Forces

Besides the size of the molecule, there is yet another factor which influences its physical relations with other molecules; this is the intensity factor in the intermolecular forces. It has already been pointed out that slight variations in the arrangement of atoms result in the most profound variations in molecular behavior as regards nearly all physical properties and we see this fact illustrated especially well in relative solubility. The striking thing about these variations in this case is that they consist of abrupt or stepwise variations from one condition to another—the various conditions being related to each other by simple proportion.

As the evidence from partition coefficients now stands it is apparent we are dealing not only with the properties of undissociated molecules, but with associated complexes and ions and hence with the chemical forces underlying association and dissociation, as well. But despite the fact that this complication precludes more direct physical-chemical consideration, it gives the results greater physical significance because it brings all these phenomena under one category without in any way invalidating the physical interpretations based upon the results.

This simple stepwise variation in behavior mentioned above is shown by a large variety of substances: compounds containing carbon, hydrogen, oxygen, nitrogen, chlorine, bromine, iodine and sulphur in a variety of chemical combinations. Hence this phenomenon cannot be attributed to peculiarities in molecular or atomic structure because it appears to be wholly independent of the kind of atoms or molecules involved. It must instead be attributed to something common to all atomic species and inherent in atomic structure itself. Undoubtedly it is to be associated with the now generally accepted idea that atoms absorb and emit energy discontinuously, in whole multiples of a unit of energy $h\nu$, or Planck's quantum. This unit has a different value for every frequency of radiation, ν, but it involves a universal constant, h. It is to be noted that this quantum is a unit of action, since it has the dimensions of energy multiplied by time, whereas the element of time does not enter into our present consideration. We are comparing various physical systems in their characteristic and essentially fixed equilibrium states.

Bohr has assumed that the electron in a hydrogen atom could revolve about the nucleus only in certain orbits which represent conditions of stable equilibrium. On the absorption or emission of a quantum of energy the electron would jump from one orbit to another and thus enter another stable state.[1]

No one has, as yet, satisfactorily explained the existence of stable states, though this conception underlies Bohr's explanation of the quantum and the theories of atomic structure as well. Thus Langmuir[2] has suggested that each electron occupied a more or less permanent position in the atom which he called a cell, the various cells being arranged in symmetrical positions around the nucleus of the atom. In discussing the properties of the electron he has said, "It seems that the electron must be regarded as a complex structure which undergoes a series of discontinuous changes while it is being bound by the nucleus or kernel of the atom."[1]

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[1] For a general discussion of the quantum theory see "The Quantum Theory," Edwin Plimpton Adams: Bull. of the National Research Council, Vol. I, Part 5, No. 5; Wm. C. McC. Lewis: "A System of Physical Chemistry," Vol. III, "Quantum Theory" (1919). Also Max Planck: Phil. Mag., **28**, 60 (1914). On the application of the quantum theory to radiation from liquids and solids see H. Stanley Allen: Ibid., **35**, 338, 404, 445 (1918).

[2] Jour. Am. Chem. Soc., **41**, 868 (1919).

Thomson[2] has considered the behavior of the electron on the hypothesis that the field of force in the immediate vicinity of the positive charge changes alternately from attraction to repulsion. At great distances these forces would reduce to the inverse square law but for atomic distances the change in sign would result in a series of positions of zero force which would be positions of stable equilibrium. Other assumptions of a more or less arbitrary nature have been made by Nicolson,[3] Brillouin,[4] Allen,[5] Lamar[6] and others to account for the discontinuous absorption and emission of energy as postulated under the quantum theory.

4. The Conservation of Energy and Inter-electronic Equilibration

The intermolecular forces are commonly supposed to have their origin in the so-called "chemical" or valence electrons. No one has advanced any very definite suggestion as to how these electrons function while holding molecules together in the liquid state. It is usually assumed that the cohesional forces are due to stray fields of force, residual affinity, etc.[7]

It does not seem possible that an electron may be so situated that its electrostatic or electro-magnetic affinities are satisfied, and that it may still be able to permanently influence, without dynamic expense, other electrons by virtue of "stray fields of force." It seems much more consistent to believe that a principle of conservation applies to all interelectronic relations, including the intermolecular forces; that the intermolecular forces are expendable by the molecule to a definite and limited extent dependent on the electronic fabrication of the molecule, just as it has always been assumed in the case of the interatomic forces. Since the intermolecular forces have their genesis in the electrons of the constituent atoms, we can arrive at such a position simply by assuming that a state of conservative equilibration exists between all the electrons which are involved in the interatomic and intermolecular forces. That is, in the atom or molecule, or in any homogeneous system of atoms or molecules, all the electrons exert forces on all the other electrons, holding each other in certain positions of stable equilibrium. If any of the physical conditions of the system are altered by the absorption or emission of energy, or by admixture with another system, certain electrons are displaced and take up new positions of equilibrium. On the assumption that energy is added to or taken from a system only by a discontinuous process, it would necessarily follow that the alteration of any system from one physical state to another would likewise be discontinuous. Various systems (or various molecular species) could be treated as a single system in various equilibrium states, and the various systems would bear definite relations to each other which would result in simple mathematical relationships between the partition coefficients, the frequencies of spectral lines and all other physical properties.

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[1] Phys. Rev., **8,** 300 (1919).

[2] J. J. Thomson: Phil. Mag., **37,** 419 (1919).

[3] J. W. Nicolson: Ibid., **28,** 90 (1914).

[4] Marce Brillouin: Comptes rendus, **168,** 1318 (1920).

[5] H. Stanley Allen: Phil. Mag., **41,** 113 (1921).

[6] Joseph Lamar., Ibid., **42,** 592 (1921).

[7] Albert P. Mathews: Jour. Phys. Chem., **20,** 554 (1916); Herbert Chatley: Proc. London Phys. Soc., **29,** 206 (1916); Irving Langmuir: Jour. Am. Chem. Soc., **39,** 1848 (1917); S. C. Bradford: Phil. Mag., **38,** 696 (1919); W. C. McC. Lewis: **28,** 104 (1914); Wm. D. Harkins and H. H. King: Jour. Am. Chem. Soc., **41,** 970 (1919).

It should be reiterated that the experimental data from partition coefficients deal with various systems in their characteristic equilibrium states, and that the element of time, a necessary consideration in the process of change from one equilibrium state to another, is not involved. In this respect, these data are unique, for they demonstrate that the existence of these discrete differences in the equilibrium states of a given system, or of various systems, is independent of the time element. The evidence warrants the assumption that these discrete differences are attributable to some property inherent in the fundamental charges themselves. Some such assumption as that made by Thomson, namely that the field of force around the fundamental charges changes alternately from attraction to repulsion, appears to offer the only explanation of the facts.

Johns Hopkins University
School of Hygiene and Public Health
Department of Physiology
Baltimore
and
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis

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